Mobile Robot Team Forming for Crystallization of Proteins

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Abstract

The process of protein crystallization is explained using the theory of robotics, particularly *path planning* of mobile robots. Path planning is a procedure which specifies motion trajectories of multiple mobile robots to form a robotic team with a desired pattern. Since protein crystals consist of a large number of protein molecules which come together to form a 3D lattice of uniform structure, it is hypothesized that each protein behaves like a mobile robot and takes adequate path to form a robotic team (crystal). Based on this hypothesis, it is shown that trajectories of the proteins should be *simple* and *local*, which generates three rules of motion for the protein robots, i.e., *a.* each protein searches for its nearest neighbor, *b.* each protein takes the shortest path to approach the nearest neighbor, and *c.* multiple proteins may form a sub-team of proteins. It is then proven mathematically that the planned path according to the three rules is stable and able to crystallize the proteins. Interaction forces at the molecular level are analyzed to show that the simple and local motion of the proteins is physically warranted. Computer simulation and experimental results are presented to validate the new theory.

Keywords

Mobile robots, robot team forming, protein crystallization, simplicity and locality.

1. Introduction

Since the completion of human genome sequencing a few years ago, a major focus of the biology research has been the determination of the structure and function of proteins which the genome codes for. This represents a huge undertaking since there are between 30,000 to 200,000 proteins in the human body, and proteins are the most structurally complicated molecules existing and functioning in an organism. The only reliable method for determining the atomic structure of a protein is x-ray crystallography ([1-2]) which requires the protein of interest to be purified and solubilized in an appropriate medium and to form a well ordered crystal through incubating. Scientists in biology have long considered crystal growing as more *art* than *science* because not a theory can completely explain the process [3], while appropriate physical and chemical conditions supporting the growth have to be discovered by exploring a huge combinatorial space, which is tedious and time consuming [4].

In the area of membrane proteins, for example, an effective method called *in meso* for crystallizing membrane proteins was introduced some years ago by Landau and Rosenbusch [5]. The basic recipe for growing crystals *in meso* includes three steps: a. combine 2 parts of protein solution/dispersion with 3 parts of lipid (monoolein), b. overlay with precipitant, and c. incubate at 20 °C. Protein crystals can then form in hours to days. The method involves combining protein with lipid and the spontaneous formation of cubic phases, and dispensing the cubic phase into a small container for mixing with precipitant and for incubation [6-7]. The ratio between the amounts of the protein and the lipid, the volume and type of the precipitant, and the temperature and duration of incubation all affect the quality of the crystal.

The process of protein crystallization poses a challenging question: how do protein molecules which are randomly distributed in the medium move to form the uniform structure? Various theories and hypotheses have been generated over the years aiming to discover optimal physical and chemical conditions which are most conducive to protein crystallization [8-10]. Researchers are still curious about the *motion trajectory* which individual protein molecules take to eventually form the crystal. Hopefully such an outstanding can help scientists reduce the time and effort in searching for the optimal conditions.

In this paper, we propose to study the motions of the proteins by a completely new approach which is *robotic team forming by proper motion trajectories of mobile robots*. The idea is inspired by research activities of two different disciplines: *biology* and

robotics. In biology, scientists are amused by important functions which proteins play in a completely autonomous way which is similar to autonomous robots working collaboratively to perform useful functions. In this regard, biologists even call proteins *nature's robots* in a recent book [11]. In robotics, robot team forming is for multiple *mobile robots* to establish a robotic pattern which is optimal for performing a given task [12-13]. By combining the two perspectives, we consider each protein as an autonomous robot, and protein crystallization as a process of robot team forming. This analogy is reasonable because crystal is a set of orderly connected proteins, and crystallization needs the proteins to form a symmetrical pattern. Our goal is to give the crystallization process a mathematical description similar to that of path planning for mobile robots such that the process has more flavor of science.

Robot team forming for performing robotic tasks has been studied by many works in recent years. Passino, Liu, and Gazi investigated control strategies for cooperative uninhabited autonomous vehicles (UAV) by modeling them as social forging swarms, and developed conditions which led multiple UAVs to cohesive foraging [14-15]. Chio and Tarn developed rules and control strategies for multiple robots to move in hierarchical formation based on a so-called four-tier hybrid architecture [16]. Some other works have used a leader-follower approach [17-19] to control the team forming in which the motion is specified for only one robot, called leader, and the others just follow. In [20], Fregene et al. developed a pursuit-evasion scheme to coordinate multiple autonomous vehicles by modeling them as classes of hybrid agents with certain level of intelligence.

Robot team forming has its root in the swarm by large number of small animals such as bees and birds, which continues to be a topic of study in recent years [21-23]. For example, Li *et al.* [21] studied stable flock of swarms using local information by which a theory of decentralized controller is proposed to explain the behavior of self-organized swarms of natural creatures. Liang and Suganthan [22] developed a new swarm algorithm to divide the whole population of individual entities into small swarms which are regrouped frequently for information exchange between the groups. For the latter purpose, Das, et al. [24] also developed an efficient communication protocol between mobile robots while they are in motion to form a team of desired pattern.

In reviewing the literature, we found that the existing strategies for robot team forming are very complicated which employ sophisticated schemes such as the Lyapunov stability theory [14-15] to control the motions of mobile robots or to explain the swarm of living agents. These strategies are not suitable for solving the current problem

because protein molecules are not able to plan complicated motions. In addition, all the proteins in the medium are equal and cannot have different rules of motion. These two practical issues impose significant constraints to any theory which can explain the crystallization process as well as be acceptable to the biology community. In this paper, we attempt to present such a theory which meets the two constraints.

We borrow the technique from robotics to develop the theory in two steps. The first step is *path planning* which defines the motion trajectories of the protein robots for forming the team (crystal). The second is robot-servoing which drives the protein robots to follow the planned path. In the first step we plan a *simple* and *local* path which is realistic for the protein robots to take, while in the second we define a control law which governs the planned motion of the protein robots, and further prove that the motion is stable. To guarantee the control law, we find a *natural force*, i.e., the so-called van der Waals force, to drive the protein robots. Our major contribution is to define a set of rules which is realistic yet effective for the protein robots, and prove that such a trajectory is naturally possible.

The paper is organized as follows. In the next section, we develop an effective path which is able to guide the proteins to form the crystal. In the third section, we examine the protein dynamics to see how the proposed motion is physically possible and stable from the control theory point of view. Simulation and experimental results are presented in the fourth section to verify our theory, which is followed by the section of conclusions.

2. Protein Path Planning for Crystallization

In this section, we first define the rules which govern the trajectory of the protein robots in the process of team forming, and then analyze the stability of the motion as well as the shape of the team formed by the protein robots.

2.1 The Model of the Motion

Path planning in robotics is to plan the motions of individual robots such that a particular pattern of the team can be formed. Using the same technique we define the path that each protein robot should take to eventually form the crystal. To solve this problem, we need to understand the intrinsic structure of crystal. A crystal is a substance which has ordered connections of its composing elements such as atom, molecule, or ion which form symmetrical 3D lattice in its structure [25]. Depending on the way and extent of symmetry, there are many kinds of systems in crystal. The most symmetrical

possible structure is called *isometric* system which comprises three crystallographic axes of equal length and at right angles to each other. Other systems are symmetrical in different ways, but all has a *uniform* structure which is the key element for the success of x-ray crystallography.

Each atom or molecule has its own structure which is isometric in many cases such as diamond and most mineral crystals. Proteins on the other hand are structurally complicated molecules, each type of which has a unique 3D shape consisting of amino acids folded or coiled into specific conformations [26], which is not isometric, i.e., not symmetric in its structure. Thus, both position and orientation of the protein robots must be right in the team forming process; otherwise, symmetrical structure of the crystal is not possible. For convenience we model each protein as a rigid body shown in Fig. 1 and limit our study to 2D. The result, however, can be extended to 3D without much difficulty.

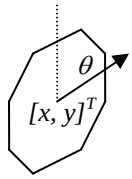


Fig. 1 Modeling the position and orientation of a protein.

We further use three parameters to describe the state of a protein robot, two for the position $[x, y]^T$ and one for the orientation θ, of the protein robot. The mass and inertia of the protein are not relevant when we discuss the kinematics of the robot, which however will be defined later when the dynamics of the protein robot is to be studied. The detailed structure of the protein, however, is not considered here.

As mentioned in Introduction, *simplicity* and *locality* are two key features for the motion of protein robots, for which we propose three rules to dictate the process of path planning of the protein robots.

***Rule 1*: Each protein searches for its nearest neighbor**. To form a team, each protein has to position itself in a correct way with respect to its team members. A protein robot is not intelligent enough to determine where many other proteins are but is assumed able to find its nearest neighbor. This assumption is reasonable because a protein robot will always be attracted to its nearest team member by the natural force existing between them. This natural force will be further discussed later in this paper.

***Rule 2*: Once the nearest neighbor is found, the protein moves towards it by taking the shortest path until a predefined distance is reached.** This rule is reasonable again as the natural force mentioned in Rule 1 drives the two molecules until it becomes null.

***Rule 3*: Multiple proteins may form a super-protein which eventually becomes crystal.** As protein robots move together following the first two rules, more and more proteins are bound together to form what we call super-protein. The super-proteins will continue attract proteins according to Rules 1 and 2 until no free proteins are available in the medium when the super-proteins become crystal. The super-proteins, however, may or may not attract each other to form a larger super-protein depending on the size of the super-protein.

The above three rules generate straightforward trajectory for the protein robots which is the shortest path towards the nearest neighbor. By such a motion, each protein robot does not move according to a *global* strategy, but only a *local* path to reach its destination until all the "natural" forces disappear and the crystal is formed. Now the question is whether the simple and local motions can make the protein robots form the desired shape of the team, i.e., the crystal. For that purpose, the following analysis is in order.

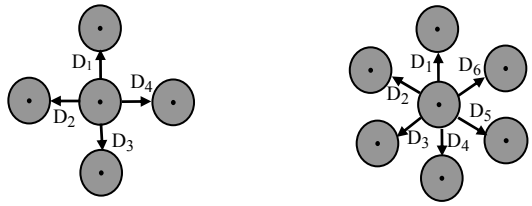
The simple and local motion of the protein robots can be described by the following equation:

$$\dot{X}_i = C[(X_{in} - X_i) - D_p] \tag{1}$$

where $X_i = [x\ y\ \theta]^T$ represents the position and orientation of the current protein, X_{in} represents the same of the nearest neighbor, and C is a coefficient which is a function of time, i.e., it varies as the two protein robots getting closer. Equation (1) reveals that when the distance between the current protein robot and its nearest neighbor is not equal to D_p, which is the desired distance between any two proteins in the crystals, the robot will keep moving until that is true. The velocity of the robot is proportional to the difference between the actual and desired distances. Note again that the mass and inertia of the protein are not involved in (1) because the analysis here is on kinematics of the robots.

The value of D_p with $p = 1, 2, 3 .. n$ depends on the structure of the crystal and so does n. For example, if the crystal has a rectangular grid as shown in Fig. 2a, $n = 4$ and each D_p is shown as a vector. For the structure as shown in Fig. 2b, $n=6$ and the D_p is significantly different from those in Fig. 2a. It is unknown in which pattern of the grid

the proteins choose to form the crystal, but the x-ray crystallography will reveal the structure of the protein so long as the grid is uniform in both position and orientation [27]. Note that in Fig. 2, only the positions of the protein robots are shown, but the orientation of all the proteins must be uniform for forming the crystal.



a b

Fig. 2 Structures of protein crystal with different D_p.

From the above discussion, one can see that each protein will have to select a D_p for its local motion which appears to violate the simplicity rule. Fortunately it is not a complicated matter because D_p is always determined by where the nearest neighbor is. That is, once the nearest neighbor is determined, D_p is automatically directed from the position of the current protein robot to that of the neighbor.

Not all the proteins move simultaneously together to form a single large crystal at once. Some of the proteins will come together to form a small group earlier than others. We call these groups super-proteins. The super-proteins will continue to attract its nearest proteins (or to attract other super-protein) to form an even large group. Such a process will continue until no free proteins are available. A super-protein may or may not attract other super-proteins to form a larger super-protein, depending on the sizes of the two super-proteins when they meet which will be further explained later.

2.2 Stability and Shape Analysis

The motion of the protein robots as defined by (1) is extremely simple. The question is if that motion is stable to generate the desired structure of the crystal, for which we need to perform stability analysis by which we want to prove that all the protein robots come to rest at constant positions based on the motion trajectory defined by (1). To do so, we take the derivative of (1) with respect to time and get the following differential equation:

$$\ddot{X}_i = \dot{C}[(X_{in} - X_i) - D_p] + C(\dot{X}_{in} - \dot{X}_i). \quad (2)$$

Without loss of generality consider X_{in} to be constant. Equation (2) can be expressed as:

$$\ddot{X}_i + C\dot{X}_i + \dot{C}X_i = \dot{C}(X_{in} - D_p). \quad (3)$$

Equation (3) now describes the motion of the protein robot assuming it takes the trajectory defined by (1) which on the other hand is hypothesized according to the three rules.

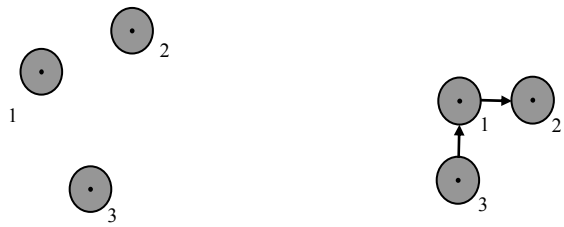
From the classical control theory [28], equation (3) represents a second order system which is unconditionally *stable* provided that $\dot{C}$ and C are positive definite. That condition can be satisfied by the inter-molecular forces, which will be shown true when we discuss the dynamics of the protein robot.

Equation (3) shows that two protein robots move towards each other to eventually bind at the desired distance D_p, which has been proved stable. It is still not convincing that massive amount of proteins taking the same type of motions will bind and eventually form the crystal. Here we will use a technique called *induction proof* to show that multiple protein robots can bind to form crystals by performing the *local* motion of (1). Induction is a powerful tool in discrete mathematics which is suitable for proving properties associated with natural numbers, *0, 1, …* [29]. If a property is true for *0* or *1*, one can assume that it is true for any number *k*. Then the induction is to show that the same property is true for number *k+1*. If the latter is successful, one can claim that the proof of the property is completed. Using the method of induction proof, we propose and prove the following theorem:

Theorem*: a number of proteins move with the local motion as defined by (1) will come together to form one or more crystals with the desired structure.*

Proof: The induction proof takes the following three steps:

Step 1. Let the first protein be numbered $n_p=1$, and the second $n_p=2$. According to (3), we obtain a single pair of proteins which are bond together. Now let $n_p=3$, i.e., there are three proteins as shown in Fig. 3a. According to the rules of motion, two closest proteins in the medium move towards each other until the desired distance (relative position and orientation) is reached. Suppose that the distance between proteins *1* and *2* is shortest, and that between *1* and *3* is next. Proteins *1* and *2* will move together regardless of the position of protein *3*, and proteins *1* and *3* will move together regardless of *2*. Consequently, the three proteins will form the group as shown in Fig. 3b.



a. b.

Fig. 3 Three proteins come together to form the team.

Step 2. Assume that *k* proteins form a super-protein.

Step 3. Let the largest $n_p = k+1$. Assume *k* of the *k+1* proteins form a super-protein before the $(k+1)^{th}$ protein. The last protein will still go to the super-protein formed by the first *k* proteins until it is bonded with the super-protein, which is also defined by the rules of the motion. It should be mentioned that multiple bindings could occur simultaneously, and all the bindings continue until protein molecules in the medium are exhausted. Therefore, multiple pieces of crystal could form at the end of the process.

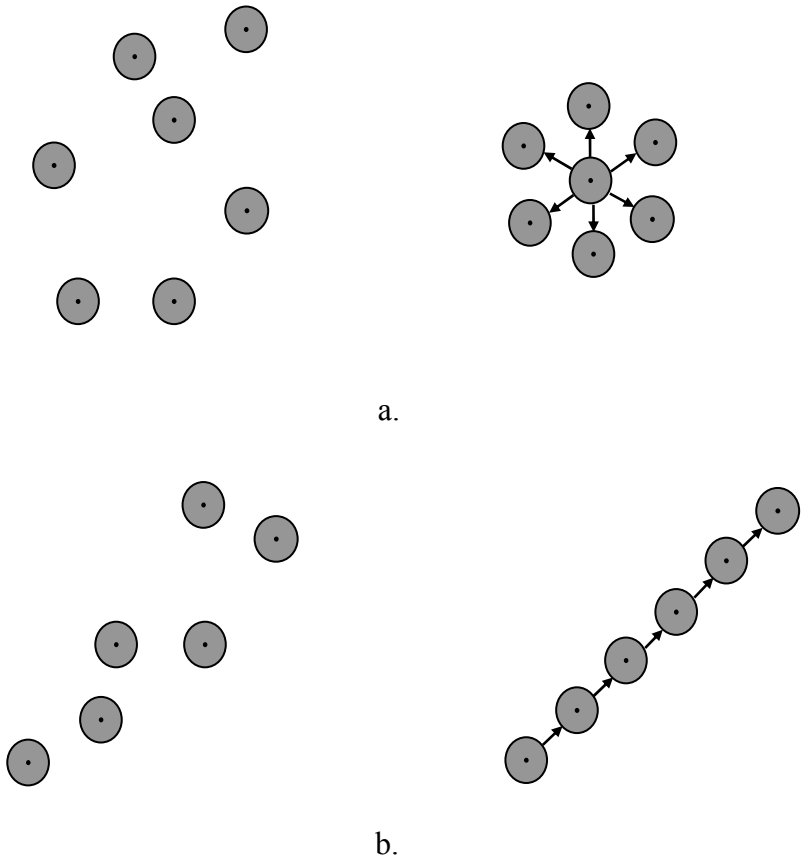
The above proof only shows that the proteins can form crystal with a uniform *structure*. It leaves the *shape* of the crystal undetermined. By using equation (1), the final shape of the crystal will depend on the distribution of the proteins in the medium space before the crystallization process starts. For example, if the proteins are evenly distributed within a circular area, the final shape is likely to be a circle or a polygon as shown in Fig. 4a. If the proteins are distributed over a long and narrow area as shown in Fig. 4b, the final shape is likely to be a stick. Then a challenging question follows: can the simple and local motions of the proteins form any shape of crystal by positioning the proteins accordingly if possible? The answer to that question is not that straightforward. We argue that some, not any, shapes can be formed by the protein robots. Consider a group of robots which are moving in the direction *N* as shown in Fig. 5a. If we limit D_p to just one possibility (Fig. 5b) and modify the three rules of motion to the following, the robots will form a line as shown in Fig. 5b.

Rule 1: Each robot searches for its nearest neighbor within its limited field of view, and becomes its follower once found; each robot has only one follower.

Rule 2: Once the nearest neighbor is found, the robot moves towards it taking the shortest path until the desired distance is reached. The robot will then maintain the distance until the team needs to be reformed for a new pattern.

Rule 3: Multiple robots may form a sub-team which may continue to attract still isolated

robots until none is available.

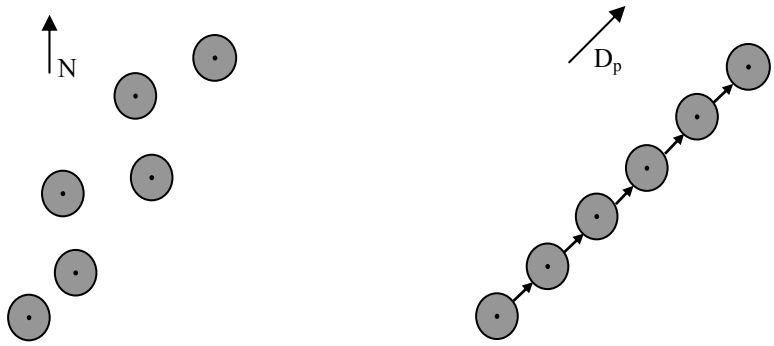


a.

b.

Fig. 4 Global shape formation of the protein robots.

Comparing the new set of rules with the earlier set, one may find that the motion of the robot has become more complicated. The nearest neighbor is no longer chosen blindly but done under certain constraints which can only be achieved by more "intelligent" organisms. Protein molecules as mentioned earlier do not possess such intelligence; therefore, the shape can only be random, depending on how the proteins are distributed in the medium. Recall the three steps for crystallizing membrane proteins which involve combining 2 parts of protein solution/dispersion with 3 parts of lipid (monoolein). How well the protein solution is mixed with the lipid may affect the distribution of the proteins in the medium, and eventually the shape of the protein crystal.



a. b.

Fig. 5 Limited local motion forms a straight line.

3. Driving Force of the Motions of Proteins in Crystallization

In the previous section we prove that simple and local motions of individual proteins can come together to form crystals of uniform structure. The idea is borrowed from the strategy of path planning in robotics. Robot path planning is an issue of kinematics which involves no mass and inertia of the robots. To guarantee the proposed motion, regardless how simple and local it could be, we still need to study the dynamics of the protein robots. In robot team forming, the driving force is regulated by some control laws reacting to the position and velocity of the robot. In protein crystallization, we can only rely on natural forces which drive the protein robots to follow the planned path. Ultimately, we need to understand how the proposed motion is achieved physically in the medium, for which the key is the interactive forces between the protein molecules. At the molecule level, the van der Waals force is the only natural force which can play such a role.

3.1 The van der Waals Force and the Local Motion

According to the basic properties of matters, we understand that there exist physical forces of *attraction* and *repulsion* between molecules which are responsible for the motion and cohesion of molecules in the forming of crystals ([25]). These forces are called van der Waals forces and act only over a relatively *short* distance between two molecules. The combined effect of the attraction and repulsion forces generates a potential which can be expressed by the following equation:

$$E_p = -\frac{A}{r_{ij}^m} + \frac{B}{r_{ij}^n} \tag{4}$$

where r_{ij} represents the distance between the two molecules, m, n, A, and B are parameters depending on the properties of the proteins and the medium which the proteins are in. It should be mentioned that the first term on the right-hand side represents the attraction potential E_{att}, and the second term the repulsion potential E_{re}. Also m is smaller than n. The combined potential of E_{att} and E_{re} can be seen in Fig. 6 which shows little repulsion force when two molecules are not close.

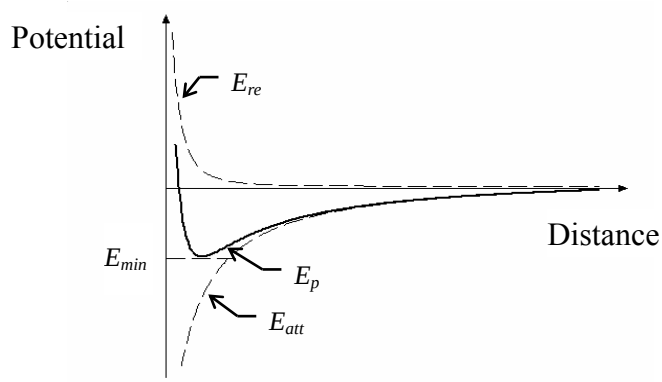


Fig. 6 van der Waals forces generate potential between two protein molecules.

In the combined curve of E_p, there is a point of minimal potential E_{min} which is called *dissociation energy*, and the distance between the two proteins at that point is called the *length of bond.* Since the potential is minimal, the molecules are forced to stay at that distance, which is similar to a spring connecting two small balls together. When the two balls move away or towards each other, the force of the spring will bring them back. Only when the potential between the two balls is minimal, i.e., at the distance which the spring produces no force, the balls remain stationary. In the protein crystallization process, protein molecules are far apart to start with, and the attraction potential dominates to bring the molecules together. The two molecules have no chance to be closer than the length of bond since the repulsion potential increases much faster than the attraction potential. Consequently, we can claim that the length of bond r_0 is identical to D_p, i.e.

$$r_0 = D_p \ . \tag{5}$$

Using D_p as the equilibrium point, the interaction force between the two protein molecules as described in Fig. 6 can be expressed as

$$F = C_{fp}[(X_{in} - X_i) - D_p] \tag{6}$$

where C_{fp} is positive definite because F monotonically increases in the positive direction when $(X_{in}\text{-}X_i)$ is greater than D_p and negative otherwise. Taking into consideration the nonlinearity of the relationship, it is reasonable to add a second order term to (6), i.e.,

$$F = C_{fp}[(X_{in} - X_i) - D_p] + C_{fv}(\dot{X}_{in} - \dot{X}_i) \tag{7}$$

where C_{fv} is also positive definite. By applying the Newton dynamic equation to the protein robot, one obtains:

$$\ddot{X}_i = m^{-1}F = m^{-1}C_{fp}[(X_{in} - X_i) - D_p] + m^{-1}C_{fv}(\dot{X}_{in} - \dot{X}_i) \tag{8}$$

where m is the mass and inertia of the protein molecule. One can see that equation (8) is equivalent to equation (2). That is, in (2) $\dot{C}$ is replaced by $m^{-1}C_{fp}$, and C is replaced by $m^{-1}C_{fv}$. Consequently, the van der Waals forces warrant a stable process of crystallization. Since protein molecules are separated and distributed in the chemical medium, only the attraction forces are effective when the crystallization process starts. Clearly, if the protein molecules are too few and too far apart, the van der Waals forces may not be large enough to bring them together. Therefore the density of the protein molecules in the medium is an important factor for successful crystallization.

It should be noted that there exists the van der Waals force between any pair of proteins so long as they are close to each other. For simplicity, we consider all the forces to be insignificant except the one from the nearest neighbor. This assumption is reasonable because the van der Waals force vanishes quickly as the distance between two proteins becomes large. Another interesting issue is what happens when two neighboring protein molecules are exactly the same distance from the current molecule. According to the rules of the motions, the current protein robot will have to make a random choice if the path is artificially planned. Naturally, two equal van der Waals forces will be applied to the protein simultaneously, and the protein will be moved by the combined force, not towards any of the two proteins. However, if there is a random factor in the medium which makes the protein a little closer to one of the two proteins, the van der Waals force from the closer one will dominate, and the one neighbor rule resumes. Since the probability of same distance is extremely small, it is not considered in the rules of motion.

The property of the van der Waals force can further explain why super-proteins may or may not attract each other. When the size of the two super-proteins is too large, the

distance between them prevents a large enough van der Waals force to attract them together. On the other hand, when the size of the super-protein is still comparable with a macromolecule [26], the distance allows a large van der Waals force which drives the two super-proteins together for binding. Furthermore, the proteins on the surfaces of the super-proteins have close contact with free proteins in the surrounding medium space and are able to attract them to the super-proteins. As a result, a super-protein is able to grow in two ways so long as free proteins in the chemical medium are not exhausted.

3.2 The Issue of Orientation

We have so far not touched the issue of orientation of the protein robots, which should all be the same for forming uniform and well-ordered structure of the crystal. For the purpose of path planning, we only need to change the second rule of the rules of motion as defined in Section 3 slightly. That is, when the nearest neighbor is found, the protein robot moves towards it taking the shortest path and aligning in the same direction as the neighbor in the three dimensional space. The question is how this can be physically achieved. Our hypothesis is that both the *polarization* of the protein and the van der Waals force play a role in the alignment.

Proteins are macro molecules consisting of a chain of amino acids which are in turn composed of multiple atoms of hydrogen, carbon, oxygen, and nitrogen, etc. A large number of electrons associated with the atoms move continuously between the atoms of the amino acids. They can pile up on one side of the protein that creates negative polarity on one end, and positive on the other known as *polarization* as shown in Fig. 7. The negative end attracts the positive end of another protein by the van der Waals forces, and vice versa. These forces create the local motions as discussed in the previous sub-section.

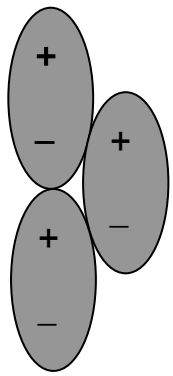


Fig. 7 Polarized proteins attract and align with each other.

In addition to the attraction force, the dipole of the proteins enables an *external electric field* to align the proteins in the same direction as the field. Thus, the orientations of all the polarized proteins, as defined by the directions of the vector which goes from the negative end to the positive as shown in Fig. 8, become identical automatically in a medium which has such an electric field. We hypothesize that the most qualified candidate for generating the electric field in the *in meso* process, for example, is the *lipid membrane* in which the membrane proteins are embedded at a natural status. Each lipid molecule has a hydrophilic head and a hydrophobic tail, and the heads attach to each other and the tails aligned on one side ([8]). In the crystallization process, the membrane proteins are washed free by certain detergent from the lipid membrane. The lipid molecules in the medium continue to generate a strong and uniform electric field to align the protein molecules in the same direction. Thus a uniform and well-ordered structure of the crystal becomes possible.

The electric field as just mentioned only forces the polarizing vector to align in the same direction as the electric field. The protein robots can still rotate about the axis of the vector as shown in Fig. 8. One needs another force to lock the protein robot at an angle which is identical to all the proteins. We consider both the structure of the protein and the van der Waals force to be responsible for this alignment. The protein as a macro-molecule has complicated structure in comparison with atoms and small molecules. Thus, when the van der Waals forces attract two proteins together, the energy is not always minimal due to the difference in the angle of rotation, which leads to an unstable binding. Only when the two protein robots are aligned correctly in all the directions, the two proteins are locked into each other to generate the minimal energy possible.

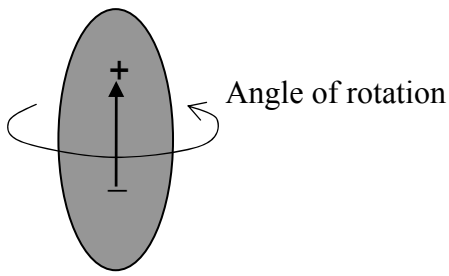


Fig. 8 The protein robot can rotate about its axis of polarized vector which is aligned with the electric field.

4. Simulation and Experimental Results

We use TeamBots, a multi-robot software, which is developed by the CMU MultiRobot Lab as the environment for simulation of protein crystallization. Each protein robot is represented by a black circle which is randomly distributed in the medium space. For simplicity, no orientation is considered in the simulation run. The protein robot is very small with a radius of *5 nm* which is similar to the size of a true protein ([26]). The length of D_p is *10 nm* in the simulation.

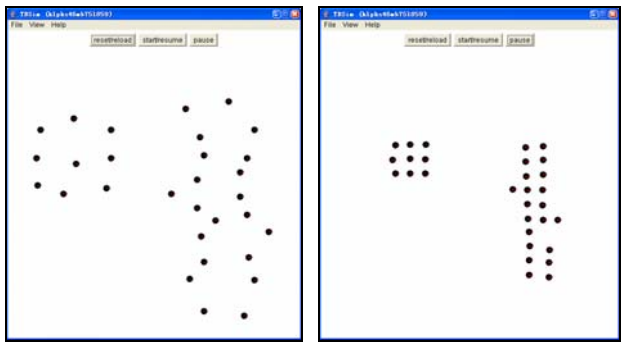
In the first simulation, *30* protein robots are randomly distributed in the mixture of protein solution/dispersion and lipid molecules along with the precipitant/salt which create the medium space. The original distance between the proteins is assumed to be between *15* to *20 nm* which is small enough for the van der Waals force to become effective.

Using equation (1), one has to select the parameter *C* which is related to the potential between two molecules. From Fig. 6, one can see that *C* increases nonlinearly as the two molecules gets closer until the two reaches the length of bond. From the curve of Fig. 6, we select the following nonlinear equation for *C*:

$$C = N[(X_{in} - X_i) - D_p]^{-3}. \tag{9}$$

Let $N = 0.05\ nm^{-3}/sec$. The proteins will move with a speed of *0.0005 nm/sec* when they are *20 nm* away from the nearest neighbor until *0.05 nm/sec* when only *11 nm* apart. The proteins will not move any longer when the bond is formed which is *10 nm*. With the above parameters, the two proteins which are *20 nm* apart take approximately *5,000 seconds* to become just *15 nm*. It will take much shorter time to travel the final *5 nm* for reaching the length of bond.

In the simulation we assume each time step to be *150* second. The *30* protein robots form two pieces of crystal in *45* steps, i.e., *6,750* seconds. That time scale is in line with the actual crystallization experiments (from hours to days). Fig. 9 shows both original and final locations of the protein robots. We can clearly see two groups of protein robots forming in the process. Once the two are formed individually, no sufficiently large force exists to drive them together, and the crystallization process stops.



a b

Fig. 9 Simulation of team forming of protein robots: a. initial distribution, and b. formation of crystals.

To see how the theoretical analysis and simulation results compare with the real crystallization process, we have examined the experimental results of the membrane protein crystallization process. The experiment was performed in the bio-chemistry laboratory at The Ohio State University which is specilized in the crystallization of membrance proteins. The experiment result is shown in Fig. 10 ([30]). One can see that pieces of the protein crystal (darker pink color) formed at the end, and some are larger than others. The result is in agreement with the theoretical analysis and the simulation results. That is, multiple pieces of protein crystal formed from a large number of protein robots when the latter takes a simple motion strategy as described in this paper.

We have also conducted a simulation for the second set of rules as mentioned in Section 2. That is, additional constraints are imposed on the simple and local motions of the protein robots. That constraints include the direction of the motion and the field of view in seeking the nearest neighbors. For the given direction of motion, and different angles of views, both shown in Fig. 11, *30* robots form different pieces of lines after *3,200* seconds as shown in Fig. 11. This result shows that with only a slight increase of the level of intelligence, a team of protein robots can form globally different shape of crystal.

5. Conclusions

In this paper, we have developed a new theory to explain the growth of protein crystal. The theory is based on the strategy of robot path planning for team forming by multiple mobile robots. *Simplicity* and *locality* are two principles in planning the path

of the protein robots. The two principles are necessary since we cannot assume proteins to have a high level of intelligence. Mathematical analysis proves that using the proposed motion strategy, proteins are attracted to its nearest neighbor until the distance of bond is reached. Physical analysis based on the van der Waals forces shows that the proposed motion is physically possible as well as stable. In addition, uniform orientation of the proteins which is a must condition for well-ordered structure can by realized by the electric field, generated by bio-chemical medium involved in the crystallization process, and the van der Waals forces collectively. The structure of the protein is also a factor of the uniform orientation.

Simulation results verify that the proposed motions of the protein robots can form crystal with a globally uniform structure. The results are in agreement with the experiments which we conducted in our laboratory in which pieces of protein crystal formed after a period of incubation in a constant environment. We further propose that the shape of the robot team or the crystal can be controlled to certain extent when some constraints are imposed to the local motions of the proteins. The latter however requires a higher level of intelligence which may not be easily achieved by alternating the medium space. Our future study should explore more the relationship between the local motions of individual components and the global shape of the organisms which the components build.

This paper represents a new attempt to study the growth of protein crystal using the robotic team forming technique. The key of the technique is the *two steps* involved, i.e., planning the motion and proving the planned motion to be stable by a control law. We hope this method can be extended to other biological agents such as cells. Those agents move autonomously as a mobile robot, but the motion trajectories could be much more complicated than the protein robots, and the force of control may not be as simple as the van der Waals force. The two-step approach, however, is still a valid tool to analyze the trajectories and cause of the motions.

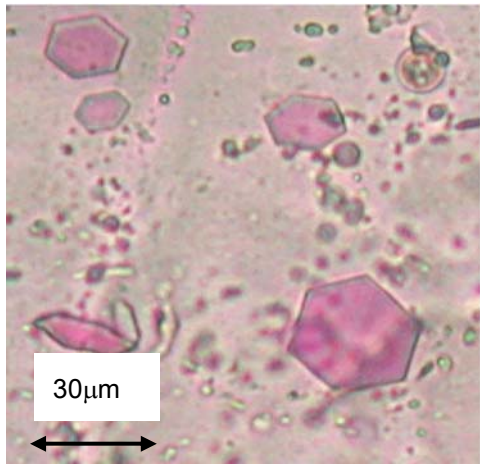


Fig. 10 Crystals of a membrane protein, bacteriorhodopsin, obtained after 3 days using 25 nL of cubic phase (100 ng of protein) in 1 μL of precipitant solution at 20 °C ([30]).

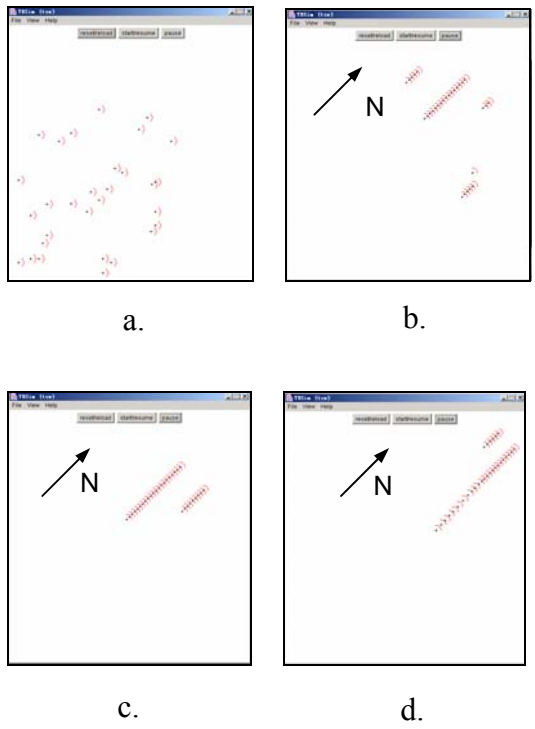


Fig. 11 Simulation of team forming of protein robots with constraints: a. initial distribution, b. angle of view=60^{o}, c. angle of view=120^{o}, and d. angle of view=180^{o}

References

1. V. Luzzati, "X-ray diffraction studies of lipid-water systems," *Biological Membranes, Physical Facts and Functions*, Vol. 1, Edited by D. Chapman, New York: Academic Press, 1968, pp. 71-123.
2. M. Caffrey, "X-ray diffraction as a technique for studying the mesomorphic phase properties of lipids," *Membranes, Metabolism and Dry Organisms,* Edited by A. Leopold, *New York: Cornell University Press*, 1986, pp. 350-357.
3. Protein Crystallization, Techniques, Strategies, and Tips, T. M. Bergfors Edts, *International University Line*, La Jolla, CA, 1999.
4. V. Cherezov, A. Peddi, L. Muthusubramaniam, Y. F. Zheng and M. Caffrey, "A robotic system for crystallizing membrane and soluble proteins in lipidic meso phases," *Acta. Cryst. D*, D60, 2004, pp. 1795-1807.
5. E. Landau and J. Rosenbusch, "Lipidic cubic phases: a novel concept for the crystallization of membrane proteins," *Proc. National Academy of Science, USA*, Vol. 93, 1996, pp. 14532-14535.
6. A. Cheng, B. Hummel, H. Qiu, and M. Caffrey, "A Simple mechanical mixer for small viscous lipid-containing samples," *Chem. Phys. Lipids*, Vol. 95, 1998, pp. 11-21.
7. V. Luzzati, "Biological significance of lipid polymorphism-the cubic phases," *Curr. Opin. Struct. Biol.*, Vol. 5, 1997, pp. 661-668.
8. M. Caffrey, "A lipid's eye view of membrane protein crystallization in mesophases," *Curr. Opin. Struct. Biol.,* Vol. 10, 2000, pp. 486-497.
9. P. ten Wolde and D. Frenkel, "Enhancement of protein crystal nucleation by critical density fluctuations," *Science*, Vol. 277, Sept. 1997, pp. 1975-1978.
10. V. Talanquer and D. Oxtoby, "Crystal nucleation in the presence of a metastable critical point," *J. of Chemical Physics*, Vol. 109, 1998, pp. 223-227.
11. C. Tanford and J. Reynolds, Nature's Robots: a History of Proteins, *Oxford University Press,* 2004.
12. L. Parker, F. Schnider, and A. Schultz, (eds.), Multi-Robot Systems: from Swarms to Intelligent Automata, *Springer,* Vol. 111, 2005.
13. X. Ceng, S. Li, and D. Xia, "Study of self-organization model of multiple mobile robots," *Int. J. of Advanced Robotic Systems*, Vol. 2, No. 3, 2005, pp. 235-238.
14. V. Gazi and K. M. Passino, "Stability analysis of social foraging swarms," *IEEE Trans. on Systems, Man, and Cybernetics-Part B: Cybernetics,* Vol. 34, No. 1, Feb. 2004, pp. 539-557.
15. Y. Liu and K. M. Passino, "Stable social foraging swarming in a noisy environment," *IEEE Trans. on Automatic Control,* Vol. 49, No. 1, Jan. 2004, pp. 30-44.

16. T.-S. Chio and T. J. Tarn, "Rules and control strategies of multi-robot team moving in hierarchical formation," *Proc. IEEE Int. Conf. on Robotics and Automation*, Taipei, Taiwan, Sep. 2003, pp. 2701-2706.

17. J. Desai, J. Ostrowski, and V. Kumar, "Controlling formations of multiple mobile robots," *Proc. IEEE Int. Conf. on Robotics and Automation,* Leuven, Belgium, May 1998, pp. 2864-2869.

18. N. Leonard and E. Fiorelli, **"**Virtual leaders, artificial potentials and coordinated control of groups," *Proc. IEEE Conf. on Decision and Control*, Orlando, FL, Dec. 2001, pp. 2968-2973.

19. J. Sweeney, H. Li, R. Grupen, and K. Ramamritham, "Scalability and schedulability in large, coordinated, distributed robot systems," *Proc. IEEE Int. Conf. on Robotics and Automation*, Taipei, Taiwan, Sep., 2003, pp. 4074-4079.

20. K. Fregene, D. Kennedy, and D. Wang, "Multi-vehicle pursuit-evasion: an agent-based framework," *Proc. IEEE Int. Conf. on Robotics and Automation*, Taipei, Taiwan, Sep., 2003, pp. 2707-2713.

21. X. Li, J. Xiao, and Z. Cai, "Stable flocking of swarms using local information," *Proc. IEEE Int. Conf. on Systems, Man and Cybernetics,* Oct. 2005, pp. 3921-3926.

22. J. Liang and P. Suganthan, "Dynamic multi-swarm particle swarm optimizer," *Proc. IEEE Swarm Intelligence Symposium*, June 2005, pp. 124-129.

23. B. Secrest and G. Lamont, "Visualizing particle swarm optimization – Gaussian particle swarm optimization," *Proc. IEEE Swarm Intelligence Symposium,* April 2003, pp. 198-204.

24. S. Das, Y. Hu, C.S. George Lee, and Y.-H. Lu, "An efficient group communication protocol for mobile robots," *Proc. IEEE Int. Conf. on Robotics and Automation,* Barcelona, Spain, April 2005, pp. 87-92.

25. T. Pollock, Properties of Matter, *McGraw-Hill, Inc.*, New York, 1995.

26. N. Campbell and J. Reece, Biology, Seventh Edition, *Addison Wesley Longman, Inc.*, 2004.

27. C. A. Taylor and H. Lipson, Optical Transforms, Their Preparation and Application to X-Ray Diffraction Problems, *Cornell University Press*, Itacha, New York, 1965.

28. J. D'Azzo, C. Houpis and S. Sheldon, Linear Control System Analysis and Design with MatLab, *Marcel Dekker, Inc.*, New York, 2003.

29. A. Meyer and R. Nagpal, Course Notes 2: Induction, from Fall'02: Mathematics for Computer Science, *MIT*, 2002.

30. L. Muthusubramaniam, A. Peddi, Y. F. Zheng, V. Cherezov and M. Caffrey, "Automating crystallization of membrane proteins by robot with soft coordinate measuring," *Proc. IEEE Int. Conference on Robotics and Automation,* New Orleans, LA, Apr. 2004, pp. 1450-1455.