COHOMOLOGY OF CRYO-ELECTRON MICROSCOPY

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Abstract. The goal of cryo-electron microscopy (EM) is to reconstruct the 3-dimensional structure of a molecule from a collection of its 2-dimensional projected images. In this article, we show that the basic premise of cryo-EM — patching together 2-dimensional projections to reconstruct a 3-dimensional object — is naturally one of Čech cohomology with \( SO(2) \)-coefficients. We deduce that every cryo-EM reconstruction problem corresponds to an oriented circle bundle on a simplicial complex, allowing us to classify cryo-EM problems via principal bundles. In practice, the 2-dimensional images are noisy and a main task in cryo-EM is to denoise them. We will see how the aforementioned insights can be used towards this end.

1. Introduction

The problem of cryo-electron microscopy (cryo-EM) asks for the following: Given a collection of noisy 2-dimensional (2D) projected images, reconstruct the 3-dimensional (3D) structure of the molecule that gave rise to these images. Viewed from a high level, it takes the form of an inverse problem similar to those in medical imaging, remote sensing, or underwater acoustics, except that for cryo-EM the data comes from an electron microscope instead of a CT scanner, radar, or sonar. However, when examined at a finer level of detail, one realizes that the cryo-EM problem possesses mathematical structures that are quite different from those of other classical inverse problems. It has inspired studies from the perspectives of representation theory [14, 15], differential geometry [37, 36], and has relations to profound problems in computational complexity [1] and operator theory [2]. This article examines the problem from an algebraic topological angle — we will show that the problem of cryo-EM is a problem of cohomology, or, more specifically, the Čech cohomology of a simplicial complex with coefficients in the Lie group \( SO(2) \) and the discrete group \( SO(2)_d \), i.e., \( SO(2) \) endowed with the discrete topology.

One might perhaps wonder about the practical value of such an abstract formulation and we would like to address this concern early on. Firstly, as we hope to convince our readers, the aforementioned cohomology framework is all very concrete and natural. The fact that cohomology has an important role to play in understanding 2D projections of 3D objects is already evident in simple examples like the Penrose tribar or Escher brick, as we will see in Section 2. Our analysis of cryo-EM data requires a more sophisticated type of cohomology but is essentially along the same lines. In fact, the same ideas that we use to study the cryo-EM problem also underlies the classical field theory of electromagnetism [6]. Secondly, this framework is useful in that it allows us to classify cryo-EM data sets: Given two different collections of 2D projected images, are they equivalent in the sense that they will give us the same 3D reconstruction? Thirdly, the insights gained would shed light on the denoising techniques: What are we really trying to achieve when we minimize a certain loss function to denoise cryo-EM images?

The technique of cryo-electron microscopy has been described in great detail in [10, 11] and more than adequately summarized in [14, 15, 29, 35, 37, 36, 38, 41, 42]. It suffices to provide a very brief review here. A more precise mathematical model, the Hadani–Singer model, for the following high-level description will be given in Section 4. The basic idea is that one first immobilizes many identical copies of a certain molecule in ice and employs an electron microscope to produce 2D images of the molecule. As each copy of the molecule is frozen in some unknown orientation, each of the 2D images may be regarded as a projection of the molecule from an unknown viewing
direction. The cryo-EM dataset is then the set of these 2D projected images. Such a 2D image shows not only the shape of the molecule in the plane of the viewing direction but also contains information about the density of the molecule, captured in the intensity of each pixel of the 2D image [27]. The ultimate goal of cryo-EM is to construct the 3D structure of the molecule from a cryo-EM dataset. In practice, these 2D images are very noisy due to various issues ranging from the electron dosage of the microscope to the structure of the ice in which the molecule are frozen. Hence the main difficulty in cryo-EM reconstruction is to denoise these 2D images by determining the true viewing directions of these noisy 2D images so that one may take averages of nearby images. There has been much significant progress toward this goal in recent years [29, 35, 38, 41, 42].

Our article attempts to understand cryo-EM datasets of 2D images via Čech and singular cohomology groups. We will see that for a given molecule, the information extracted from its 2D cryo-EM images determines a cohomology class of a two-dimensional simplicial complex. Furthermore, each of these cohomology classes corresponds to an oriented circle bundle on this simplicial complex. We note that there are essentially two interpretations of cohomology: obstruction and moduli. On the one hand, a cohomology group quantifies the obstruction from local to global. For example, this is the sense in which cohomology is used when demonstrating the non-existence of an impossible figure [31] or in the solution of the Mittag-Leffler problem [13]. On the other hand, a cohomology group may also be used to describe a collection of mathematical objects, i.e., it serves as a moduli space for these objects. For example, when we use a cohomology group to parameterize all divisors or all line bundles on an algebraic variety [16], it is used in this latter sense.

The line bundles example is a special case of a more general statement: A cohomology group serves as the moduli space of principal bundles over a topological space. This forms the basis for our use of cohomology in the cryo-EM reconstruction problem — as a moduli space for all possible cryo-EM datasets. Obviously, such a classification of cryo-EM datasets necessarily comes under the implicit assumption that the 2D images in a dataset are all noise-free. Our classification depends on a mathematical model\(^1\) for molecules in the context of cryo-electron microscopy under a noise-free assumption. In practice, when our images are noisy, this model gives us a natural way, namely, the cocycle condition, to denoise them by fitting them to the model. Various methods for denoising cryo-EM images [35, 38] may be viewed as nonlinear regression for fitting the cocycle condition under additional assumptions.

### 2. Cohomology and 2D Projections of 3D Objects

The idea that cohomology arises whenever one attempts to analyze 2D projections of 3D objects was first pointed out by Roger Penrose, who proposed in [31] a cohomological argument to analyze Escher-type optical illusions. In the following, we present Penrose’s elegantly simple example since it illustrates some of the same principles that underly our more complicated use of cohomology in cryo-EM.

We follow the spirit of Penrose’s arguments in [31] but we will deviate slightly to be more in-line with our later discussions of cryo-EM. We also provide more details including an explicit proof of nonexistence. The definitions of the few unavoidable topological jargons may be found in Section 3 but they are used in such a way that one could grasp the intuitive ideas involved even without knowledge of the jargons. To be clear, by a 3D object, we mean one that can be embedded in \(\mathbb{R}^3\).

The Penrose tribar is defined to be a (non-existent) 3D object obtained by gluing three rectangular solid cuboids (i.e., bars) \(L_1, L_2, L_3\) in \(\mathbb{R}^3\) in the following way: \(L_i\) is glued to \(L_j\) by identifying

\(^1\)The reader is reminded that a molecule is a physical notion and not a mathematical one. A mathematical answer to the question ‘What is a molecule?’ depends on the context. In one theory, a molecule may be a solution to a Schrödinger PDE (e.g., quantum chemistry) whereas in another, it may be a path in a 6\(N\)-dimensional phase space (e.g., molecular dynamics). In the Hadani–Singer model, a molecule is a real-valued function on \(\mathbb{R}^3\) representing potential.
Figure 1. (a) Projection of tribar into an annulus \( Q \subseteq \mathbb{R}^2 \). (b) Decomposition of tribar into three overlapping pieces in \( \mathbb{R}^3 \).

A cubical portion \( L_{ij} \) at one end of \( L_i \) with a cubical portion \( L_{ji} \) at one end of \( L_j \) as depicted in Figure 1(b), \( i, j = 1, 2, 3 \).

The tribar is more commonly shown in its 2D projected form as in Figure 1(a). Let \( \Delta \) be the triangular 2D object in Figure 1(a), which clearly exists in \( \mathbb{R}^2 \) or we would not have been able to draw it. Indeed, there are (infinitely) many 3D objects that, when projected onto a plane \( H \cong \mathbb{R}^2 \), gives \( \Delta \) as an image. An example is the object in Figure 2, as we explain below.

Let \( H \subseteq \mathbb{R}^3 \) be a hyperplane, which partitions \( \mathbb{R}^3 \) into two half-spaces. Let \( O \in \mathbb{R}^3 \) be an arbitrary point in one half-space and the three bars \( L_1, L_2, L_3 \) be in the other. The reader should think of \( O \) as the position of the viewer and the viewing direction as a normal to \( H \). Now we are going to arrange \( L_1, L_2, L_3 \) in such a way that their projections onto \( H \) give us \( \Delta \). This is clearly possible; for example, the 3D object in Figure 2, upon an appropriate rotation dependent on \( H \) and \( O \), would give \( \Delta \) as a projection.

Furthermore, we will draw an annulus \( Q \) around \( \Delta \) as in Figure 1(a). Henceforth we may regard \( \Delta \) as being embedded in \( Q \), instead of in \( H \), and is a projection of some 3D object onto \( Q \). In general, any nonsimply connected region (e.g. a punctured plane) could play the role of \( Q \) but it is essential that \( Q \) not be simply connected so that its cohomology is nontrivial.

Define \( d_{ij} \in \mathbb{R}^+ \) to be the distance from \( O \) to the center of \( L_{ij} \) and \( d_{ii} = 1 \), \( i, j = 1, 2, 3 \). Let \( g = (g_{ij})_{i,j=1}^{3} \) be the \( 3 \times 3 \) matrix of cross ratios

\[
g_{ij} = \frac{d_{ij}}{d_{ji}}, \quad i, j = 1, 2, 3.
\]

Then \( g \) is a matrix with \( g_{ij}^{-1} = g_{ji} \) and \( g_{ii} = 1 \) for all \( i, j = 1, 2, 3 \).

Note that the matrix \( g \) is a function of the positions of the bars \( L_1, L_2, L_3 \). These have a certain degree of freedom: We may move each of the bars independently along the viewing direction and this would keep their projections in \( Q \) invariant, always forming \( \Delta \). But moving \( L_i \) in the viewing direction results in a rescaling of the distance \( d_{ij} \) by a factor \( g_i \in \mathbb{R}^+ \) for all \( j \neq i \), i.e., if \( d'_{ij} \) denotes the new distance upon moving \( L_i \)'s along viewing directions, then \( d'_{ij} = d_{ij}/g_i \), for all \( i \neq j \). Let \( g' = (g'_{ij})_{i,j=1}^{3} \) be the new matrix of cross ratios upon moving \( L_i \)'s along viewing direction. Then we have

\[
g'_{ij} = \frac{d'_{ij}}{d'_{ji}} = \frac{d_{ij}/g_i}{d_{ji}/g_j} = g_{ij} \frac{g_j}{g_i}, \quad i, j = 1, 2, 3.
\]

(1)

Suppose that we could eventually move \( L_1, L_2, L_3 \) to form the tribar in \( \mathbb{R}^3 \). Then, in this final position, the centers of \( L_{ij} \) and \( L_{ji} \) coincide and so \( d'_{ij} = d'_{ji} \) for all \( i \neq j \), and thus \( g'_{ij} = 1 \) for all
In other words, the matrix $g$ must be a coboundary, i.e.,

$$g_{ij} = \frac{g_i}{g_j}$$

for some $g_i, g_j \in \mathbb{R}^+, i, j = 1, 2, 3$.

In summary, what we have shown is that if $L_1, L_2, L_3$ could be moved into place to form a tribar, then for $L_1, L_2, L_3$ in any positions that form $\Delta$ upon projection onto $Q$, the corresponding matrix $g$ must be a coboundary, i.e., satisfies (2). With this observation, we can get a contradiction showing that the tribar does not exist. Let $L_1, L_2, L_3$ be arranged as in Figure 2 and recall that their projections onto $Q$ give $\Delta$. In this case, the matrix $g$ is

$$g = \begin{bmatrix} 1 & 1 & 1 \\ 1 & 1 & g_{23} \\ 1 & g_{32} & 1 \end{bmatrix}.$$ 

If the tribar exists, then $g$ is a coboundary, i.e., (2) has a solution for some $g_i, g_j \in \mathbb{R}^+, i, j = 1, 2, 3$, and so

$$g_1 = g_2 = g_3,$$

implying $g_{23} = 1$. However, as is evident from Figure 2, $L_{23}$ does not even intersect $L_{32}$ and so $g_{23} \neq 1$, a contradiction.

Although the tribar does not exist as a 3D object, i.e., it cannot be embedded in $\mathbb{R}^3$, it clearly exists as an abstract geometrical object (a cubical complex) defined by the gluing procedure described earlier — we will call this the intrinsic tribar to distinguish it from the nonexistent 3D object. In fact, the intrinsic tribar can be embedded in a three-dimensional manifold $\mathbb{R}^3/\mathbb{Z}$, a quotient space of $\mathbb{R}^3$ under a certain action of the discrete group $\mathbb{Z}$ related to Figure 2 (see [9] for details).

We emphasize that a tribar is a geometrical object, not a topological one. It may be tempting to draw a parallel between the non-embeddability of the intrinsic tribar in $\mathbb{R}^3$ with the non-embeddability of the Möbius strip in $\mathbb{R}^2$ or the Klein bottle in $\mathbb{R}^3$. But these are different phenomena. As a topological object, a Möbius strip is only defined up to homotopy, i.e., we may freely deform a Möbius strip continuously. However the definition of the tribar does not afford this flexibility, i.e., a tribar is not homotopy invariant. For instance, we are not allowed to twist or bend the bars. In fact, had we allowed such continuous deformation, the intrinsic tribar is homotopy equivalent to a torus and therefore trivially embeddable in $\mathbb{R}^3$. This is much like our study of cryo-EM, where the goal is to reconstruct the 3D structure of a molecule precisely, and not just up to homotopy.

The discussions above also apply to other impossible objects in $\mathbb{R}^3$. For example, the Escher brick, defined as the (nonexistent) 3D object obtained by gluing four bars $L_1, L_2, L_3, L_4$ as in
Figure 3. If the Escher brick exists in $\mathbb{R}^3$, then whenever $L_1, L_2, L_3, L_4$ projects onto $Q$ to form Figure 3(a), the matrix $g \in \mathbb{R}^{4 \times 4}$ is necessarily a coboundary, i.e., satisfies $g_{ij} = g_i/g_j$ for some $g_i \in \mathbb{R}^+, i, j = 1, 2, 3, 4$. We may construct an analogue of Figure 2 whereby we glue three of the four ends in Figure 3(b). This 3D object projects onto $Q$ to form Figure 3(a) but its corresponding matrix $g \in \mathbb{R}^{4 \times 4}$ is not a coboundary. Hence the Escher brick does not exist in $\mathbb{R}^3$.

3. SINGULAR COHOMOLOGY AND ČECH COHOMOLOGY

This article is primarily intended for an applied and computational mathematics readership. For readers unfamiliar with algebraic topology, this section provides in one place all the required definitions and background material, kept to a bare minimum of just what we need for this article.

We will define two types of cohomology groups associated to a topological space $X$ and a topological group $G$ that will be useful for our study of the cryo-EM problem: $H^n(X, G)$, the singular cohomology group with coefficient in $G$; and $\check{H}^n(X, G)$, the Čech cohomology group with coefficient in the topological group $G$. For a given $X$, these cohomology groups are in general different; but they would always be isomorphic for the space $X$ that we construct from cryo-EM data (see Section 4). The reason we need both of them is that they are good for different purposes: the cohomology of cryo-EM is most naturally formulated in terms of Čech cohomology; but singular cohomology is more readily computable and facilitates our explicit calculations.

Our descriptions in the next few subsections are highly condensed, but in principle complete and self-contained. While this material is standard, our goal here is to make them accessible to practitioners by limiting the prerequisite to a few rudimentary definitions in point set topology and group theory. We provide pointers to standard sources at the beginning of each subsection.

We use $X \simeq Y$ to denote isomorphism if $X, Y$ are groups, homotopy equivalence if $X, Y$ are topological spaces, and bundle isomorphism if $X, Y$ are bundles. We use $X \cong Y$ to denote homeomorphism of topological spaces.

3.1. SINGULAR COHOMOLOGY. Standard references for this section are [17, 24, 39].

The standard $n$-simplex for $n = 0, 1, 2, 3$, is the set

$$\Delta_n := \{(t_0, \ldots, t_n) \in \mathbb{R}^{n+1} : \sum_{i=0}^{n} t_i = 1, t_i \geq 0\}.$$
\( \Delta_n \) is the convex hull of its \( n + 1 \) vertices,
\[
e_0 = (0, 0, \ldots, 0), \ e_1 = (1, 0, \ldots, 0), \ldots, e_n = (0, 0, \ldots, 1).
\]
The standard 0-simplex is a point, the standard 1-simplex is a line, the standard 2-simplex is a triangle, and the standard 3-simplex is a tetrahedron.

For \( n = 0, 1, 2 \), the convex hull of any \( n \) vertices \( e_{i_1}, \ldots, e_{i_n} \) of \( \Delta_n \), where \( 0 \leq i_1 < \cdots < i_n \leq n \), is called a face of \( \Delta_n \) and denoted by \([i_1, \ldots, i_n]\).

Let \( X \) be a topological space and \( n = 0, 1, 2, 3 \). A continuous map \( \sigma : \Delta_n \to X \) is called a singular simplicial simplex on \( X \). We denote by \( C_n(X) \) the free abelian group generated by all singular simplicial simplices on \( X \). The boundary maps are homomorphisms of abelian groups
\[
\partial_1 : C_1(X) \to C_0(X), \quad \partial_2 : C_2(X) \to C_1(X), \quad \partial_3 : C_3(X) \to C_2(X),
\]
defined respectively by the linear extensions of
\[
\partial_1(\sigma) = \sigma|[1] - \sigma|[0], \quad \partial_2(\sigma) = \sigma|[1,2] - \sigma|[0,2] + \sigma|[0,1], \quad \partial_3(\sigma) = \sigma|[1,2,3] - \sigma|[0,2,3] + \sigma|[0,1,3] - \sigma|[0,1,2].
\]
Here \( \sigma|[i] \) denotes the restriction of \( \sigma \) to the face \([i]\) of \( \Delta_1 \), \( \sigma|[i,j] \) denotes the restriction of \( \sigma \) to the face \([i,j]\) of \( \Delta_2 \), and \( \sigma|[i,j,k] \) denotes the restriction of \( \sigma \) to the face \([i,j,k]\) of \( \Delta_3 \). We set \( \partial_0 : C_0(X) \to \{0\} \) to be the zero map.

The sequence of homomorphisms of abelian groups
\[
C_3(X) \xrightarrow{\partial_3} C_2(X) \xrightarrow{\partial_2} C_1(X) \xrightarrow{\partial_1} C_0(X) \xrightarrow{\partial_0} 0
\]
forms a chain complex, i.e., it has the property that
\[
\partial_0 \circ \partial_1 = 0, \quad \partial_1 \circ \partial_2 = 0, \quad \partial_2 \circ \partial_3 = 0
\]
which are easy to verify. For \( n = 0, 1, 2 \), let \( Z_n(X) := \ker \partial_n \subseteq C_n(X) \) be the subgroup of \( n \)-cycles and \( B_n(X) := \text{im} \partial_{n+1} \subseteq C_n(X) \) be the subgroup of \( n \)-boundaries. It follows from (4) that \( B_n(X) \subseteq C_n(X) \). The quotient group
\[
H_n(X) := Z_n(X)/B_n(X)
\]
is called the \( n \)th singular homology group of \( X \), \( n = 0, 1, 2 \).

For \( n = 0, 1, 2, 3 \), define \( C^n(X) = \text{Hom}_{\mathbb{Z}}(C_n(X), \mathbb{Z}) \), the set of all group homomorphisms from \( C_n(X) \) to \( \mathbb{Z} \). \( C^n(X) \) is clearly an abelian group itself under addition of homomorphisms. The sequence of homomorphisms of abelian groups
\[
0 \xrightarrow{\partial_0} C^0(X) \xrightarrow{\partial_1} C^1(X) \xrightarrow{\partial_2} C^2(X) \xrightarrow{\partial_3} C^3(X)
\]
forms a cochain complex, i.e., it has the property that
\[
\partial_1^* \circ \partial_0^* = 0, \quad \partial_2^* \circ \partial_1^* = 0, \quad \partial_3^* \circ \partial_2^* = 0,
\]
which follows from (4). For \( n = 0, 1, 2 \), let \( Z^n(X) := \ker \partial_n^* \subseteq C^n(X) \) be the subgroup of \( n \)-cocycles and \( B^n(X) := \text{im} \partial_{n+1}^* \subseteq C^n(X) \) be the subgroup of \( n \)-coboundaries. The quotient group
\[
H^n(X) := Z^n(X)/B^n(X)
\]
is called the \( n \)th singular cohomology group of \( X \), \( n = 0, 1, 2 \). More generally, let \( G \) be a group then one can define the \( n \)th singular cohomology group \( H^n(X, G) \) with coefficient \( G \) of \( X \) to be the cohomology groups \( Z^n(X, G)/B^n(X, G) \) of the cochain complex
\[
0 \xrightarrow{\partial_0} C^0(X, G) \xrightarrow{\partial_1} C^1(X, G) \xrightarrow{\partial_2} C^2(X, G) \xrightarrow{\partial_3} C^3(X, G)
\]
where $C^n(X, G) = \text{Hom}_Z(C_n(X), G)$, $\partial_n^*$ is the map induced by $\partial_n : C_n(X) \to C_{n-1}(X)$, $n = 0, 1, 2$ and

\[
Z^n(X, G) := \text{Ker} \partial_{n+1}^* \subseteq C^n(X, G), \\
B^n(X, G) := \text{Im} \partial_n^* \subseteq C^n(X, G).
\]

Note that when $G = \mathbb{Z}$, $C^n(X, \mathbb{Z}) = C^n(X)$, $Z^n(X, \mathbb{Z}) = Z^n(X)$, $B^n(X, \mathbb{Z}) = B^n(X)$, $H^n(X, \mathbb{Z}) = H^n(X)$.

For the purpose of this paper, $X$ would take form of a finite simplicial complex, a collection $K$ of finitely many simplices such that

(i) every face of a simplex in $K$ is also contained in $K$;
(ii) the intersection of two simplices $\Delta_1, \Delta_2$ in $K$ is a face of both $\Delta_1$ and $\Delta_2$.

We denote the union of simplices in $K$ by $|K|$. We also say that a topological space $X$ is a finite simplicial complex if $X$ can be realized as $|K|$ for some finite simplicial complex $K$. For example, spheres $S^n$ and tori $S^1 \times \ldots \times S^1$ are finite simplicial complexes in this more general sense.

For the purpose of this paper, readers only need to know that

\[
H_0(S^2) \simeq H_2(S^2) \simeq \mathbb{Z}, \quad H_1(S^2) = 0, \quad H^0(S^2) \simeq H^2(S^2) \simeq \mathbb{Z}, \quad H^1(S^2) = 0,
\]

and that if $X$ is a simplicial complex of dimension $p$ then $H_n(X) = 0$ for all $n > p$.

A topological space $X$ is contractible if there is a point $x_0 \in X$ and a continuous map $H : X \times [0, 1] \to X$ such that

\[
H(x, 0) = x_0 \quad \text{and} \quad H(x, 1) = x.
\]

Roughly speaking, this means that $X$ can be continuously shrunk to a point $x_0$. For example, an open/closed/half-open-half-closed line segment is contractible, as is an open/closed disk or a disk with an arc on the boundary. The following is the only fact about contractible spaces that we need for this article.

**Proposition 3.1.** If $X$ is contractible and $G$ is an abelian group, then $H^n(X, G) = 0$ for all $n > 0$ and $H^0(X, G) = G$.

### 3.2. Principal bundles and classifying spaces

Standard references for this section are [17, 19, 24, 25, 39].

Let $G$ be a group with multiplication map $\mu : G \times G \to G$, $(x, y) \mapsto xy$ and inversion map $\iota : G \to G$, $x \mapsto x^{-1}$. If $G$ is also a topological space such that $\mu$ and $\iota$ are continuous then $G$ together with this topology is called a topological group. Every group $G$ is a topological group if we put the discrete topology on $G$; we will denote such a topological group by $G_d$ (unless the natural topology is the discrete topology, in which case we will just write $G$). For example, $\mathbb{Z}$ with its natural discrete topology is a topological group. In this article, we are primarily interested in the case where $G$ is the group of $2 \times 2$ real orthogonal matrices. When endowed with the manifold topology, this is $SO(2)$, the special orthogonal group in dimension two and is homeomorphic to the unit circle $S^1$ as topological spaces. On the other hand, $SO(2)_d$ is just a discrete uncountable collection of $2 \times 2$ orthogonal matrices. Both $SO(2)$ and $SO(2)_d$ will be of interest to us.

Let $X, P, F$ be topological spaces. We say that $\pi : P \to X$ is a fiber bundle with fiber $F$ and base space $X$ if every point of $X$ has a neighborhood $U$ such that $\pi^{-1}(U)$ is homeomorphic to $U \times F$. In particular, $\pi^{-1}(x) \cong F$ for all $x \in X$.

A principal $G$-bundle is a tuple $(P, \pi, \varphi)$ where $\pi : P \to X$ is a fiber bundle with fiber $G$ and $\varphi : G \times P \to P$ is a group action such that

(i) $\varphi$ is a continuous map;
(ii) $\varphi(g, f) \in \pi^{-1}(x)$ for any $f \in \pi^{-1}(x)$;
(iii) if $\varphi(g, f) = f$, then $g$ is the identity element in $G$;
(iv) For any $x, y$ and $f_x \in \pi^{-1}(x), f_y \in \pi^{-1}(y)$, there is a $g \in G$ such that $\varphi(f_x) = f_y$.  
We will often say ‘P is a principal G-bundle on X’ to mean the above, without specifying π and ϕ. A principal \( SO(2) \)-bundle is called an oriented circle bundle and a principal \( SO(2)_d \)-bundle is called a flat oriented circle bundle. We will have more to say about these in Sections 4 and 5.

Let \((P, π, ϕ)\) and \((P′, π′, ϕ′)\) be two principal G-bundles on X. We say that \((P, π, ϕ)\) is isomorphic to \((P′, π′, ϕ′)\), denoted \( P \cong P′ \), if there is a homeomorphism \( θ : P → P′ \) compatible with the group actions \( ϕ, ϕ′ \) and the projection maps \( π, π′ \) in the following sense:

\[
θ \circ ϕ = ϕ′ \circ (id_G × θ) \quad \text{and} \quad π' \circ θ = π.
\]

Here \( id_G : G → G \) is the identity map. If \( U = \{U_i : i ∈ I\} \) is an open covering of X such that \( π^{-1}(U_i) \cong U_i × G \) via some isomorphism \( π_i \) for all \( i ∈ I \). A transition function corresponding to \( U \) is a map \( τ_{ij} := τ_iτ_j^{-1} \), defined for all \( i, j ∈ I \) such that \( U_i ∩ U_j ≠ ∅ \). It may be regarded as a G-valued function \( τ_{ij} : U_i ∩ U_j → G \). Transition functions are important because one may construct a principal G-bundle entirely from its transition functions [19].

For \( G = SO(2) \), transition functions \( τ_{ij} \) of an oriented circle bundle are continuous \( SO(2) \)-valued functions on open sets \( U_i ∩ U_j \). For \( G = SO(2)_d \), transition functions \( τ'_{ij} \) of a flat oriented circle bundle are continuous \( SO(2)_d \)-valued functions on open sets \( U_i ∩ U_j \) but since \( SO(2)_d \) has the discrete topology, this means that \( τ'_{ij} \) are locally constant \( SO(2) \)-valued functions on \( U_i ∩ U_j \). In particular, if \( U_i ∩ U_j \) is connected, then \( τ'_{ij} \) are constant \( SO(2) \)-valued functions on \( U_i ∩ U_j \). In our case, the covering that we choose (see (13)) will have connected \( U_i ∩ U_j \)'s and so we may regard

\[
\{ \text{isomorphism classes of flat oriented circle bundles} \}
\subseteq \{ \text{isomorphism classes of oriented circle bundles} \}.
\]

In other words, flat oriented circle bundles are just oriented circle bundles whose transition functions are constant valued.

Let X, Y be topological spaces. Two maps \( h_0, h_1 : X → Y \) are homotopic if there is a continuous function \( H : X × I → Y \) such that

\[
H(x, 0) = h_0(x) \quad \text{and} \quad H(x, 1) = h_1(x).
\]

Homotopy is an equivalence relation and the set of homotopy equivalent classes of maps from X to Y is denoted by \([X, Y]\). Let \( S^n \) be the n-sphere. We say that a topological space X is weakly contractible if \([S^n, X]\) contains only the trivial element. The classifying space of a topological group G is a topological space BG together with a principal G-bundle EG on BG such that EG is weakly contractible.

**Proposition 3.2.** For any topological space X and topological group G, there is a one-to-one correspondence between the following two sets:

\([X, BG] \leftrightarrow \{ \text{isomorphism classes of principal G-bundles on X} \},\)

given by \( h ↦ h^*(EG) \), the principal G-bundle on X whose fiber over \( x ∈ X \) is the fiber of EG over \( h(x) ∈ BG \).

For the purpose of this paper, readers only need to know that the classifying space \( BU(n) \) of the unitary group \( U(n) \) is \( \text{Gr}(n, ∞) \), the Grassmannian of n-planes in \( \mathbb{C}^∞ \). In particular, if \( n = 1 \), since \( U(1) = SO(2) \), we have

\[
BSO(2) = \mathbb{C}P^∞.
\]  

(7)

Let G be an abelian group with identity 0. We write \( \text{Hom}_Z(G, Z) \) for the set of all homomorphisms from G to Z. An element \( g ∈ G \) is a torsion element if it has finite order, i.e., \( n \cdot g = 0 \) for some \( n ∈ \mathbb{N} \). The subgroup of all torsion elements in G is called its torsion subgroup and denoted \( G_T \). For example, every element in \( \mathbb{Z}/m\mathbb{Z} \) is a torsion element whereas 0 is the only torsion element in \( \mathbb{Z} \). For an abelian group G, we also denote its torsion subgroup as

\[
G_T = \text{Ext}_1^Z(G, Z).
\]
The reason for including this alternative notation is that it is very standard — a special case of something defined more generally [17, 18]. We now state some routine relations [18] that we will need for our calculations. Let $G$ and $G'$ be abelian groups. Then

\[ \text{Hom}_\mathbb{Z}(G_T, \mathbb{Z}) = 0, \quad \text{Hom}_\mathbb{Z}(G/G_T, \mathbb{Z}) \simeq G/G_T, \quad \text{Hom}_\mathbb{Z}(G \oplus G', \mathbb{Z}) \simeq \text{Hom}_\mathbb{Z}(G, \mathbb{Z}) \oplus \text{Hom}_\mathbb{Z}(G', \mathbb{Z}), \]

\[ \text{Ext}^1_\mathbb{Z}(G_T, \mathbb{Z}) = G_T, \quad \text{Ext}^1_\mathbb{Z}(G/G_T, \mathbb{Z}) = 0, \quad \text{Ext}^1_\mathbb{Z}(G \oplus G', \mathbb{Z}) \simeq \text{Ext}^1_\mathbb{Z}(G, \mathbb{Z}) \oplus \text{Ext}^1_\mathbb{Z}(G', \mathbb{Z}). \]

Singular homology and singular cohomology are related via $\text{Ext}^1_\mathbb{Z}$ and $\text{Hom}_\mathbb{Z}$ in the following well-known theorem.

**Theorem 3.3** (Universal Coefficient Theorem). Let $X$ be a topological space. Then we have a natural short exact sequence

\[ 0 \to \text{Ext}^1_\mathbb{Z}(H_1(X), \mathbb{Z}) \to H^2(X) \to \text{Hom}_\mathbb{Z}(H_2(X), \mathbb{Z}) \to 0. \]

In particular we have an isomorphism,

\[ H^2(X) \simeq \mathbb{Z}^b \oplus T_1, \]

where $b := \text{rank}(H_2(X)) = b_2(X)$ is the second Betti number of $X$ and $T_1$ is the torsion subgroup of $H_1(X)$.

The second Betti number of $X$ counts the number of 2-dimensional ‘voids’ in $X$. In the case of interest to us, where $X$ is a finite two-dimensional simplicial complex, the second Betti number counts the number of 2-spheres (by which we meant the boundary of a 3-simplex, which is homeomorphic to $\mathbb{S}^2$) contained in $X$.

We will also need the following alternative characterization [24, Chapter 22] of $H^2(X)$.

**Theorem 3.4.** Let $X$ be a topological space. Then we have

\[ [X, \mathbb{C}P^\infty] \simeq H^2(X). \]

### 3.3. Čech cohomology

Standard sources for this are [13, 16, 21].

Let $G$ be a topological group and let $X$ be a topological space. For any open subset $U$ of $X$ we define an assignment

\[ U \mapsto \mathcal{G}(U) := \text{group of } G\text{-valued continuous functions on } U \]

for all open subset $U \subseteq X$. By definition, if $G$ is a discrete group and $U$ is any connected open subset of $X$, then $\mathcal{G}(U) = G$. If $U \subseteq V$ then we have a restriction map

\[ \rho_{V,U} : \mathcal{G}(V) \to \mathcal{G}(U) \]

defined by the restriction $G$-valued continuous functions on $V$ to $U$.

Let $X$ be a topological space and $G$ be a topological group on $X$. Let $\mathcal{U} = \{U_i : i \in I\}$ be an open covering of $X$. We may associate a cochain complex to $X$, $G$, and $\mathcal{U}$ as follows:

\[ C^0(\mathcal{U}, G) \xrightarrow{\delta_0} C^1(\mathcal{U}, G) \xrightarrow{\delta_1} C^2(\mathcal{U}, G) \]

(8)

where

\[ C^0(\mathcal{U}, G) = \prod_{i \in I} G(U_i), \]

\[ C^1(\mathcal{U}, G) = \left\{ (g_{ij})_{i,j \in I} \in \prod_{i,j \in I} G(U_i \cap U_j) : g_{ij} g_{ji} = 1 \text{ for all } i,j \in I \right\}, \]

\[ C^2(\mathcal{U}, G) = \left\{ (g_{ijk})_{i,j,k \in I} \in \prod_{i,j,k \in I} G(U_i \cap U_j \cap U_k) : g_{ijk} g_{ikj} = g_{ijk} g_{kji} = g_{ijk} g_{jik} = 1 \text{ for all } i,j,k \in I \right\}, \]

and

\[ (\delta_0(g_i))_{j,k} = g_k g_j^{-1}, \quad \text{for all } j,k \in I, \]

\[ (\delta_1(g_{ij}))_{k,l} = g_{lm} g_{mk} g_{kl}, \quad \text{for all } k,l,m \in I. \]
To be precise, we have
\[ g_k g_j^{-1} = \rho_{U_k, U_k \cap U_j} (g_k) \cdot \rho_{U_j, U_k \cap U_j} (g_j^{-1}), \]
\[ g_{lm} g_{mk} g_{kl} = \rho_{U_l, U_l \cap U_m} \rho_{U_m, U_m \cap U_k} \rho_{U_k, U_k \cap U_l} (g_{lm}) \cdot \rho_{U_k, U_l \cap U_k} \rho_{U_l, U_k \cap U_l} (g_{mk}) \cdot \rho_{U_k, U_l \cap U_k} \rho_{U_k, U_l \cap U_k} (g_{kl}). \]

It is easy to check that \( \delta_1 \circ \delta_0 = 0 \) and so (8) indeed forms a cochain complex.

As in the case of singular cohomology, the set \( \tilde{B}^1 (\mathcal{U}, G) := \text{Im} \delta_0 \) and \( \tilde{Z}^1 (\mathcal{U}, G) := \text{Ker} \delta_1 \) are the subsets of \( \check{C}ech \) 1-coboundaries and \( \check{C}ech \) 1-cocycles respectively. Again we have \( \tilde{B}^1 (\mathcal{U}, G) \subseteq \tilde{Z}^1 (\mathcal{U}, G) \). The first \( \check{C}ech \) cohomology group associated to \( \mathcal{U} \) with coefficient in \( G \) is then defined to be the quotient group
\[ \tilde{H}^1 (\mathcal{U}, G) := \tilde{Z}^1 (\mathcal{U}, G) / \tilde{B}^1 (\mathcal{U}, G). \]

Explicitly, we have
\[ \tilde{H}^1 (\mathcal{U}, G) = \{ (g_{ij}) : g_{ij} g_{jk} g_{kl} = 1 \text{ for all } i, j, k \} / \{ (g_{ij}) : g_{ij} = g_j g_i^{-1} \text{ for all } i, j \}. \]

We have in fact already encountered this notion in Section 2, \( \tilde{H}^1 (Q, \mathbb{R}^+) \), the \( \check{C}ech \) cohomology group of the annulus \( Q \) with coefficients in the group \( \mathbb{R}^+ \) has appeared implicitly in our discussion.

By its definition, \( \tilde{H}^1 (\mathcal{U}, G) \) depends on the choice of open covering \( \mathcal{U} \) of \( X \). To obtain a \( \check{C}ech \) cohomology group of \( X \) independent of open covering, we take the direct limit over all possible open coverings of \( X \). The first \( \check{C}ech \) cohomology group of \( X \) with coefficient in \( G \) is defined to be the direct limit
\[ \check{H}^1 (X, G) := \varprojlim \tilde{H}^1 (\mathcal{U}, G) \]
with \( \mathcal{U} \) running through all open coverings of \( X \).

For those unfamiliar with the notion of direct limit, \( \check{H}^1 (X, G) \) may be defined explicitly using an equivalence relation:
\[ \check{H}^1 (X, G) := \left[ \bigsqcup_{\mathcal{U}} \tilde{H}^1 (\mathcal{U}, G) \right] / \sim, \]
where \( \bigsqcup_{\mathcal{U}} \) denotes the disjoint union of \( \tilde{H}^1 (\mathcal{U}, G) \) for all possible open coverings of \( X \). The equivalence relation \( \sim \) is given as follows: For \( \varphi_{\mathcal{U}} \in \tilde{H}^1 (\mathcal{U}, G) \) and \( \varphi_{\mathcal{V}} \in \tilde{H}^1 (\mathcal{V}, G) \), \( \varphi_{\mathcal{U}} \sim \varphi_{\mathcal{V}} \) iff

(i) there is an open covering \( \mathcal{W} \) such that every open set \( W \in \mathcal{W} \) is contained in \( U \cap V \) for some \( U \in \mathcal{U} \) and \( V \in \mathcal{V} \);

(ii) there is an element \( \varphi_{\mathcal{W}} \in \tilde{H}^1 (\mathcal{W}, G) \) such that the restriction\(^2 \) of \( \varphi_{\mathcal{U}} \) and the restriction of \( \varphi_{\mathcal{V}} \) are both equal to \( \varphi_{\mathcal{W}} \).

As the reader can guess, calculating the \( \check{C}ech \) cohomology group using such a definition would in general be difficult. Fortunately, the following theorem (really a special case of Leray’s theorem \([8]\)) allows us to simplify the calculation in all cases of interest to us in this article.

**Theorem 3.5** (Leray’s theorem). Let \( X \) be a topological space and \( G \) be an abelian topological group. Let \( \mathcal{U} = \{ U_i : i \in I \} \) be an open cover of \( X \) such that \( \check{H}^1 (U_i, G) = 0 \) for all \( i \in I \). Then we have
\[ \check{H}^1 (\mathcal{U}, G) \simeq \check{H}^1 (X, G). \]

Furthermore, we will often be able to reduce calculation of \( \check{C}ech \) cohomology to calculation of singular cohomology since they are equal in the case when \( X \) is a finite simplicial complex \([32]\).
Theorem 3.6. If $K$ is a finite simplicial complex and $G$ is an abelian group, then
\[ \check{H}^1(K, G_d) \simeq H^1(K, G), \]
where $G_d$ is the group $G$ equipped with the discrete topology.

For a contractible space, we have $H^1(K, G) = 0$ by Proposition 3.1. So we may deduce the following from Theorem 3.6.

Corollary 3.7. If $K$ is a finite contractible simplicial complex and $G$ is an abelian topological group, then
\[ \check{H}^1(K, G) = 0. \]

To check whether an oriented circle bundles on a finite simplicial complex $K$ is flat, we have the following useful result [22, 26, 28].

Proposition 3.8. An oriented circle bundle on $K$ is flat if and only if it corresponds to a torsion element in $H^2(K)$.

A particularly important result [5, 20] for us is the following theorem that relates the Čech cohomology group with $G$-coefficients and principal $G$-bundles.

Theorem 3.9. $\check{H}^1(X, G)$ is in canonical one-to-one correspondence with the set of isomorphism classes of principal $G$-bundles on $X$.

4. Cohomological classification of discrete cryo-EM data

We will follow the mathematical setup for the cryo-EM problem as laid out in [14, 15]. First recall the high-level description of the problem: Given data comprising a collection of noisy 2D projected images, reconstruct the 3D structure of the molecule that gave rise to these images. The Hadani–Singer model casts the problem in mathematical terms and may be described as follows:

(i) The molecule is described by a function $\varphi : \mathbb{R}^3 \to \mathbb{R}$, the potential function of the molecule.
(ii) A viewing direction is described by a point on the 2-sphere $S^2$.
(iii) The position of an image is described by a $3 \times 3$ matrix $A = [a, b, c] \in SO(3)$ where the orthonormal column vectors $a, b, c$ are such that span$\{a, b\}$ is the projection plane and $c$ is the viewing direction.
(iv) A projected image $\psi$ of the molecule $\varphi$ by $A$ is described by a function $\psi : \mathbb{R}^2 \to \mathbb{R}$ where
\[ \psi(x, y) = \int_{z \in \mathbb{R}} \varphi(xa + yb + zc) \, dz. \]

The function $\psi$ describes the density of the molecule along the chosen viewing direction.

Let $\Psi = \{\psi_1, \ldots, \psi_n\}$ be a set of $n$ projected images of the molecule and $c_1, \ldots, c_n$ be the corresponding viewing directions. It is common to impose two mild assumptions:

(a) The function $\varphi$ is generic, i.e., each image $\psi_i \in \Psi$ has a uniquely determined viewing direction.
(b) The viewing directions $c_1, \ldots, c_n \in S^2$ are distributed uniformly on $S^2$.

In addition, since each image $\psi_i$ is associated with a viewing direction $c_i$, we should regard $\psi_i$ to be a real-valued function on the tangent plane to $S^2$ with unit normal in the direction of $c_i$. This is the point-of-view adopted in [38] and we will assume it throughout this article.

Henceforth, by a ‘molecule,’ we will mean one in the Hadani–Singer model, i.e., a function $\varphi$. These include $\varphi$’s that do not correspond to any actual molecules. We assume that $\varphi \in L^2(\mathbb{R}^3)$ and $\psi_1, \ldots, \psi_n \in L^2(\mathbb{R}^2)$. There is a natural notion of distance [29] between projected images $\Psi = \{\psi_1, \ldots, \psi_n\}$ given by
\[ d(\psi_i, \psi_j) = \min_{g \in SO(2)} \|g \cdot \psi_i - \psi_j\|, \]
where \( \| \cdot \| \) is the norm in \( L^2(\mathbb{R}^2) \) and the action of \( g \in SO(2) \) on a projected image \( \psi \) is
\[
(g \cdot \psi)(x, y) = \psi(g^{-1}(x, y)).
\]

Geometrically, the action of \( g \) on \( \psi \) is the rotation of \( \psi \) by the angle represented by \( g \in SO(2) \). Let \( g_{ij} \) be the element in \( SO(2) \) which realizes the minimum of the distance \( d(\psi_i, \psi_j) \), i.e.,
\[
g_{ij} := \arg \min_{g \in SO(2)} \| g \cdot \psi_i - \psi_j \| \tag{9}
\]
for \( i, j = 1, \ldots, n \). Clearly, we have
\[
g_{ii} = 1_n \quad \text{and} \quad g_{ij} g_{ji} = 1, \tag{10}
\]
for all \( i, j = 1, \ldots, n \), where \( 1_n \) is the \( n \times n \) identity matrix. We will call
\[
D := \{ g_{ij} \in SO(2) : i, j = 1, \ldots, n \}
\]
the **cryo-EM data set**. This is of course derived from the raw image data set \( \Psi \) and the process of extracting \( D \) from \( \Psi \) is itself an actively research topic \([1, 2]\), particularly when the images \( \psi_i \)'s are noisy. We will not concern ourselves with this auxiliary problem here.

For any \( \varepsilon > 0 \), the Hadani–Singer model associates an undirected graph \( G_\varepsilon = (V, E) \) where \( V = \{[1], \ldots, [n]\} \) is the set of vertices\(^3\) corresponding to the projected images \( \Psi = \{\psi_1, \ldots, \psi_n\} \), and \( E \) is the set of edges defined by
\[
[i, j] \in E \quad \text{if and only if} \quad d(\psi_i, \psi_j) \leq \varepsilon. \tag{12}
\]

Let us first consider an ideal situation where the projected images \( \psi_i \)'s are noiseless. Also we fix \( \varepsilon > 0 \) and the number of images \( n \). Let \( G_\varepsilon \) be the associated undirected graph. We define the **cryo-EM complex** \( K_\varepsilon \) as follows:

(i) the 0-simplices of \( K_\varepsilon \) are the vertices of \( G_\varepsilon \),
(ii) the 1-simplices of \( K_\varepsilon \) are the edges of \( G_\varepsilon \),
(iii) the 2-simplices of \( K_\varepsilon \) are the triangles \([i, j, k]\) such that \([i, j], [i, k], [k, l]\) are all edges of \( G_\varepsilon \).

\( K_\varepsilon \) is a two-dimensional finite simplicial complex. It is the **2-clique complex** \([3, 23]\) of the graph \( G_\varepsilon \). In addition, \( K_\varepsilon \) is also the **Vietoris–Rips complex** \([7, 43]\) defined by (12) with respect to the metric \( d \).

Some simple examples: The graph \( G_1 = (V_1, E_1) \) with \( V_1 = \{[1], [2], [3]\} \) and \( E_1 = \{[1, 2], [1, 3], [2, 3]\} \) defines a simplicial complex \( K_1 \) that is a triangle. The graph \( G_2 = (V_2, E_2) \) with \( V_2 = \{[1], [2], [3], [4]\} \) and \( E_2 = \{[1, 2], [1, 3], [2, 3], [1, 4], [2, 4], [3, 4]\} \) defines a simplicial complex \( K_2 \) that is the boundary of a tetrahedron or 3-simplex. The graph \( G_3 = (V_3, E_3) \) with \( V_3 = \{[1], [2], [3], [4]\} \) and \( E_3 = \{[1, 2], [2, 3], [1, 4], [3, 4]\} \) defines a simplicial complex \( K_3 \) that is the boundary of a square.

![Diagram of simplicial complexes](image)

We will regard our simplicial complex \( K_\varepsilon \) as being embedded in \( \mathbb{R}^4 \) and inherits the Euclidean topology from \( \mathbb{R}^4 \), i.e., \( K_\varepsilon \) is a geometric simplicial complex and not just an abstract simplicial complex. For each vertex \([i]\) of \( K_\varepsilon \) we define an open set \( U_i(K_\varepsilon) \) to be the union of the interior of all simplices of \( K_\varepsilon \) containing the vertex \([i]\). Those familiar with simplicial complex might like to

\(^3\)We will use notations consistent with those introduced in Section 3.1 for simplices.
note that \( U_i(K_\varepsilon) \) is just the complement of the link of \([i]\) in the star of \([i]\). For example, \( U_i(K_i) \) for \( i = 1, 2, 3 \) are shown below. Here dashed lines are excluded from the neighborhood.

It follows from our definition of \( U_i(K_\varepsilon) \) that

\[
U = \{ U_i : [i] \text{ is a vertex of } K_\varepsilon \} \tag{13}
\]

is an open covering of \( K_\varepsilon \).

Let \( \varphi \) be a fixed molecule and \( \Psi = \{ \psi_1, \ldots, \psi_n \} \) be a set of projected images of \( \varphi \). The cryo-EM data set \( D = \{ g_{ij} \in SO(2) : i, j = 1, \ldots, n \} \) contains all \( g_{ij} \)'s corresponding to every pair of images \( \psi_i, \psi_j \). For the purpose of cryo-EM reconstruction, one does not usually need the full cryo-EM data set [38], only a much smaller subset comprising the \( g_{ij} \)'s corresponding to images \( \psi_i, \psi_j \) that are near each other, i.e., \( d(\psi_i, \psi_j) \leq \varepsilon \) for some small \( \varepsilon > 0 \). This is expected since most reconstruction methods proceed by aggregating local information. With this in mind, we define the following.

**Definition 4.1.** Let \( D = \{ g_{ij} \in SO(2) : i, j = 1, \ldots, n \} \) be a cryo-EM data set. Let \( \varepsilon > 0 \) and \( K_\varepsilon \) be the cryo-EM complex. The **discrete cryo-EM data set on** \( K_\varepsilon \) **is the subset of** \( D \) **corresponding to edges in** \( K_\varepsilon \) **given by**

\[
z_\varepsilon^d := \{ g_{ij} \in SO(2) : [i, j] \in K_\varepsilon \}.
\]

We may view \( z_\varepsilon^d \) as the ‘useful’ part of the cryo-EM data set \( D \) for cryo-EM reconstruction. In fact we are unaware of any reconstruction method that makes use of \( g_{ij} \) where \([i, j] \notin K_\varepsilon \).

As we mentioned earlier in this section, we take the point-of-view in [38] that the projected images \( \psi_i \)'s lie in tangent planes of a two-sphere determined by their viewing directions. We also assume, as in [38], that if the images \( \psi_i, \psi_j, \) and \( \psi_k \) have viewing directions close enough, then they lie in the same tangent plane. Under this assumption, one may use the geometry of \( \mathbb{R}^2 \) to show [38] that the corresponding \( g_{ij} \)'s satisfy the following 1-cocycle condition:

\[
g_{ij}g_{jk}g_{ki} = 1. \tag{14}
\]

Here 1 is the identity matrix in \( SO(2) \). As we pointed out earlier, the matrices \( g_{ij} \)'s in the cryo-EM data set must satisfy (10). The preceding discussion implies that for \( \varepsilon > 0 \) small enough, they must also satisfy (14) for all edges \([i, j], [j, k], [k, i]\) of the cryo-EM complex \( K_\varepsilon \). Given an open subset \( U \) of \( K_\varepsilon \), any element \( g \in SO(2) \) can be regarded as the constant \( SO(2) \)-valued function sending every point \( x \in U \) to \( g \), and thus we may regard \( z_\varepsilon^d \) as a cocycle in \( \tilde{Z}^1(K_\varepsilon, SO(2)_d) \). We highlight this observation as follows:

*Every discrete cryo-EM data set on* \( K_\varepsilon \) *is a Čech 1-cocycle on* \( K_\varepsilon \).

Henceforth we will regard

\[
\tilde{Z}^1(K_\varepsilon, SO(2)_d) = \{ \text{all discrete cryo-EM data sets on } K_\varepsilon \}.
\]

The set on the right includes all possible discrete cryo-EM data sets on \( K_\varepsilon \) corresponding to all molecules \( \varphi \). A cocycle \( z_\varepsilon^d \) only tells us how to glue together local information. It is possible for two different 3D molecules to give the same discrete cryo-EM data set \( z_\varepsilon^d \) as long as the relations between their projected images are the same.
Given a discrete cryo-EM data set $z^d_\varepsilon \in \hat{Z}^1(K_\varepsilon, SO(2)_d)$, i.e., elements in $z^d_\varepsilon$ satisfy (14), and any arbitrary image $\psi \in L^2(\mathbb{R}^2)$, we may apply each $g \in z^d_\varepsilon$ to $\psi$ to obtain a set of images

$$z^d_\varepsilon(\psi) := \{ g \cdot \psi : g \in z^d_\varepsilon \} = \{ g_{ij} \cdot \psi : [i,j] \in K_\varepsilon \}.$$ 

The cocycle condition (14) ensures that for any image $g \cdot \psi$ in this set, we obtain the same set of images by applying each $g \in z^d_\varepsilon$, i.e.,

$$z^d_\varepsilon(g \cdot \psi) = z^d_\varepsilon(\psi) \quad \text{for any } g \in z^d_\varepsilon.$$

Moreover, the discrete cryo-EM data set obtained would be exactly $z^d_\varepsilon$. A set of projected images $z^d_\varepsilon(\psi)$ allows one to reconstruct the 3D molecule $\varphi$ whose projected images are precisely the ones in $z^d_\varepsilon(\psi)$ [10, 11, 33, 30]. Put in another way, given a discrete cryo-EM data set $z^d_\varepsilon \in \hat{Z}^1(K_\varepsilon, SO(2)_d)$ and an image $\psi \in L^2(\mathbb{R}^2)$, we may construct a 3D molecule $\varphi \in L^2(\mathbb{R}^3)$ whose discrete cryo-EM data set is exactly $z^d_\varepsilon$ and one of whose projected image is $\psi$.

The context for the following theorem is that we are given two collections of $n$ projected images $\Psi = \{ \psi_1, \ldots, \psi_n \}$ and $\Psi' = \{ \psi'_1, \ldots, \psi'_n \}$ of the same molecule $\varphi$. These give two discrete cryo-EM data sets $D = \{ g_{ij} \in SO(2) : [i,j] = 1, \ldots, n \}$ and $D' = \{ g'_{ij} \in SO(2) : [i,j] = 1, \ldots, n \}$. Let $\varepsilon > 0$ be sufficiently small and $z^d_\varepsilon = \{ g_{ij} \in SO(2) : [i,j] \in K_\varepsilon \}$, $z'_d = \{ g'_{ij} \in SO(2) : [i,j] \in K_\varepsilon \}$ be the corresponding discrete cryo-EM data sets on $K_\varepsilon$.

**Theorem 4.2 (Bundle Classification of Cryo-EM Data I).** Let $\varepsilon > 0$ be small enough so that (14) holds and let $K_\varepsilon$ be the corresponding cryo-EM complex. Then

(i) the 1-cocycle $z^d_\varepsilon$ determines a flat oriented circle bundle on $K_\varepsilon$;

(ii) two 1-cocycles $z^d_\varepsilon$ and $z'^d_\varepsilon$ for the same molecule determine isomorphic flat oriented circle bundles if and only if

$$g'_{ij} = g_{ij}g_i g_j^{-1}$$

for some $g_i, g_j \in SO(2), [i,j] \in K_\varepsilon$.

**Proof.** Let $\mathcal{U} = \{ U_i(K_\varepsilon) : i = 1, \ldots, n \}$ be the open cover defined in (13). It is easy to see that $U_i(K_\varepsilon)$ is contractible and so by Corollary 3.7,

$$\hat{H}^1(U_i(K_\varepsilon), SO(2)_d)) = \{ 1 \}$$

for all $i = 1, \ldots, n$. We may then apply Theorem 3.5 to get

$$\hat{H}^1(\mathcal{U}, SO(2)_d) \simeq \hat{H}^1(K_\varepsilon, SO(2)_d).$$

Therefore it follows from Theorem 3.9 that $\hat{H}^1(\mathcal{U}, SO(2)_d)$ is canonically in one-to-one correspondence with the set of isomorphism classes of $SO(2)_d$-principal bundles, i.e., flat oriented circle bundles. Since the subset $z^d_\varepsilon = \{ g_{ij} \in SO(2) : [i,j] \in K_\varepsilon \}$ is a 1-cocycle in $\hat{H}^1(\mathcal{U}, SO(2)_d)$, it determines an oriented circle bundle over $K_\varepsilon$. Part (ii) follows from the fact that the 1-cocycle $b_\varepsilon = \{ g_i g_j^{-1} \in SO(2) : [i,j] \in K_\varepsilon \}$ is a 1-coboundary and thus represents the trivial cohomology class. \hfill \Box

If the reader finds (15) familiar, that is because we have seen a similar version (1) in our discussion of the Penrose tribar. The difference here is that the quantities in (1) are from the group $\mathbb{R}^+$ whereas the quantities in (15) are from the group $SO(2)$. Two cocycles $z^d_\varepsilon = \{ g_{ij} \in SO(2) : [i,j] \in K_\varepsilon \}$ and $z'^d_\varepsilon = \{ g'_{ij} \in SO(2) : [i,j] \in K_\varepsilon \}$ are said to be **cohomologically equivalent** if and only if they differ by a coboundary $b_\varepsilon = \{ g_i g_j^{-1} \in SO(2) : [i,j] \in K_\varepsilon \}$ in the sense of (15). Cohomologically equivalent $z^d_\varepsilon$ and $z'_\varepsilon$ define the same **cohomology class** in the quotient group and we have

$$\hat{H}^1(K_\varepsilon, SO(2)_d) := \hat{Z}^1(K_\varepsilon, SO(2)_d) / \hat{B}^1(K_\varepsilon, SO(2)_d)$$

$$= \{ \text{cohomologically equivalent discrete cryo-EM data sets on } K_\varepsilon \}.$$
By Proposition 3.2, the cohomology group $\tilde{H}^1(K_\varepsilon, SO(2)_d)$ can be identified as sets with the classifying space $[K_\varepsilon, BSO(2)_d]$, which classifies the isomorphism classes of flat oriented circle bundles on $K_\varepsilon$. We obtain a canonical one-to-one correspondence

$$\{\text{cohomologically equivalent discrete cryo-EM data sets on } K_\varepsilon\}$$

$$\longleftrightarrow \{\text{isomorphism classes of flat oriented circle bundles on } K_\varepsilon\}. \quad (16)$$

Finally we arrive at the following result.

**Theorem 4.3.** Let $\varepsilon > 0$ be small enough so that (14) holds and let $K_\varepsilon$ be the corresponding cryo-EM complex. Then

(i) every flat oriented circle bundle on $K_\varepsilon$ is the trivial circle bundle;

(ii) all discrete cryo-EM data sets on $K_\varepsilon$ are coboundaries $b_\varepsilon = \{g_ig_j^{-1} \in SO(2) : [i, j] \in K_\varepsilon\}$.

**Proof.** By Proposition 3.8, it suffices to show that $H^2(K_\varepsilon)$ is torsion free. But this follows from Theorem 3.3, observing that $H^1(K_\varepsilon) = 0$ by the construction of $K_\varepsilon$ and so $T_1 = 0$. \hfill \Box

In other words, the set on the right of (16) is a singleton comprising only the trivial bundle. Consequently, discrete cryo-EM data sets on $K_\varepsilon$ are all cohomologically equivalent and all correspond to the trivial circle bundle. So Theorem 4.2 does not provide a useful classification. The reason is that a discrete cryo-EM data set as defined by (9), i.e., an element of $\tilde{H}^1(K_\varepsilon, SO(2)_d)$, is too coarse. In the next section, we will see how this can be remedied by looking at continuous cryo-EM data sets.

5. **Cohomological classification of continuous cryo-EM data**

In the Hadani–Singer model, a projected image is a function $\psi : \mathbb{R}^2 \rightarrow \mathbb{R}$ defined by

$$\psi(x, y) = \int_{z \in \mathbb{R}} \varphi(xa + yb + zc) dz,$$

where $A = [a, b, c] \in SO(3)$ describes the orientation of the molecule in $\mathbb{R}^3$ and $\varphi$ is the potential function of the molecule. For every pair of images $\psi_i, \psi_j$ we define an $SO(2)$-valued function

$$h_{ij}(x, y) := \arg\min_{g \in SO(2)} \int_0^{2\pi} |(g \cdot \psi_i)(r \cos \theta, r \sin \theta) - \psi_j(r \cos \theta, r \sin \theta)|^2 d\theta, \quad (17)$$

where $r = \sqrt{x^2 + y^2}$. For any $(x, y) \in \mathbb{R}^2$, using the fact that $h_{ij}(x, y)$ depends only on the restriction of $\psi_i, \psi_j$ on the circle of radius $r = \sqrt{x^2 + y^2}$, we see\(^4\) that $h_{ij}$’s satisfy the 1-cocycle condition

$$h_{ij}(x, y)h_{jk}(x, y)h_{ki}(x, y) = 1, \quad (18)$$

if the images $\psi_i, \psi_j, \text{and } \psi_k$ have viewing directions near one another (so that we may regard them to be on the same tangent plane; see our discussion before (14)).

Recall that we write $G(U)$ for the set of $G$-valued functions on an open set $U$. So $h_{ij} \in SO(2)(\mathbb{R}^2)$.

Let $\varepsilon > 0$ be small enough so that (19) holds and let $K_\varepsilon$ be the corresponding cryo-EM complex. Let $U$ be the open covering of $K_\varepsilon$ in (13). We will now define a continuous cryo-EM data set, a Čech 1-cocycle

$$\zeta^\varepsilon := \{\tau_{ij} \in SO(2)(U_i \cap U_j) : [i, j] \in K_\varepsilon\}.$$  

on $K_\varepsilon$ determined by the $h_{ij}$’s. The process is analogous to how we obtained $z^\varepsilon$, the discrete cryo-EM data set on $K_\varepsilon$, from the cryo-EM data set $D$ in Section 4 but is a little more involved.

\(^4\)Let $\psi(r)$ be the restriction of the image $\psi$ to the circle of radius $r$ and let $h_{ij}(r) = h_{ij}(x, y)$ if $\sqrt{x^2 + y^2} = r$. To rotate $\psi_i(r)$ to $\psi_j(r)$ by $h_{ik}(r)$, we can first rotate $\psi_i(r)$ to $\psi_j(r)$ by $h_{ij}(r)$ and then rotate $\psi_j(r)$ to $\psi_k(r)$ by $h_{jk}(r)$. By the geometry of $\mathbb{R}^2$, the two ways of rotating $\psi_i(r)$ to $\psi_k(r)$ must lead to the same result, i.e., (18) must hold.
We first define the restriction of $\tau_{ij}$ to $U_i \cap U_j \cap U_k$ for all $k = 1, \ldots, n$ and show that we can glue them together to obtain a globally defined $SO(2)$-valued function on $U_i \cap U_j$. By construction, the open covering $\mathcal{U}$ has the property that for any $U_i, U_j, U_k$, either

$$U_i \cap U_j \cap U_k = \emptyset \quad \text{or} \quad U_i \cap U_j \cap U_k \cong \mathbb{R}^2.$$ 

In the first case there is nothing to define. If $U_i \cap U_j \cap U_k \cong \mathbb{R}^2$, we fix a homeomorphism and regard $U_i \cap U_j \cap U_k$ as $\mathbb{R}^2$, then define the restriction of $\tau_{ij}$ to be

$$\tau_{ij}(x, y) = h_{ij}(x, y),$$

for $(x, y) \in U_i \cap U_j \cap U_k$ and $h_{ij} \in SO(2)(U_i \cap U_j)$. Since $U_i \cap U_j \cap U_k$ is disjoint from $U_i \cap U_j \cap U_l$ whenever $k$ and $l$ are distinct, so to define $\tau_{ij}$ on $U_i \cap U_j$ we only need to define it on the set

$$V_{ij} := U_i \cap U_j - \bigcup_{k \neq i, j} U_i \cap U_j \cap U_k.$$ 

If $V_{ij} \neq \emptyset$, then it must be the interior of the 1-simplex connecting $[i]$ and $[j]$. In this case we define $\tau_{ij}$ to be the constant $^5 \lim_{r \to \infty} \tau_{ij}(x, y) \in SO(2)$ where $(x, y) \in U_i \cap U_j \cap U_k$ and $r = \sqrt{x^2 + y^2}$. Lastly, it is obvious from its definition that $\tau_{ij}$ satisfies the 1-cocycle condition

$$\tau_{ij}(x, y)\tau_{jk}(x, y)\tau_{ki}(x, y) = 1. \quad (19)$$

Since $z_\varepsilon^c$ satisfies (19), we see that $z_\varepsilon^c \in \tilde{Z}^1(K_\varepsilon, SO(2))$. By an argument similar to the proof of Theorem 4.2, we obtain the following classification result.

**Theorem 5.1** (Bundle Classification of Cryo-EM Data II). Let $\varepsilon > 0$ be small enough so that (19) holds and let $K_\varepsilon$ be the corresponding cryo-EM complex. Then

(i) the 1-cocycle $z_\varepsilon^c$ determines an oriented circle bundle on $K_\varepsilon$;

(ii) two 1-cocycles $z_\varepsilon^c$ and $z_\varepsilon^{c'}$ for the same molecule determine isomorphic oriented circle bundles if and only if

$$\tau'_{ij} = \tau_{ij} \tau_{i}^{-1} \tau_{j}^{-1} \quad (20)$$

for some $\tau_i \in SO(2)(U_i), \tau_j \in SO(2)(U_j), [i, j] \in K_\varepsilon$.

For small enough $\varepsilon > 0$, Theorem 5.1 gives us a classification of all possible continuous cryo-EM data sets on $K_\varepsilon$, a canonical correspondence

$$\{\text{cohomologically equivalent cryo-EM data sets on } K_\varepsilon\} \longrightarrow \{\text{isomorphism classes of oriented circle bundles on } K_\varepsilon\}. \quad (21)$$

By Proposition 3.2, the isomorphism classes of principal $G$-bundles may be identified with $[K_\varepsilon, BG]$, the homotopy classes of continuous maps from $K_\varepsilon$ to the classifying space of $G$. In our case, $G = SO(2) \cong S^1$, the circle group. By (7), $BG = BSO(2) \cong CP^\infty$ and so

$$\tilde{H}^1(K_\varepsilon, SO(2)) \simeq [K_\varepsilon, BSO(2)] \simeq [K_\varepsilon, CP^\infty] \simeq H^2(K_\varepsilon). \quad (22)$$

where the last isomorphism is by Theorem 3.4. We will discuss the two main implications of (22) separately: $\tilde{H}^2(K_\varepsilon)$ tells us about any obstruction to cryo-EM reconstruction; whereas $[K_\varepsilon, BSO(2)]$ tells us about the moduli space of cryo-EM data sets.

---

$^5$The limit exists because $\tau_{i,j}(x, y)$ depends only on $r = \sqrt{x^2 + y^2}$ and $SO(2)$ is compact.
5.1. Cohomology as obstruction. The cohomology group $H^2(K_ε)$ may be viewed as the obstruction to $K_ε$ degenerating into a one-dimensional simplicial complex. If $H^2(K_ε) = 0$, then $K_ε$ contains no 2-sphere and $K_ε$ is a two-dimensional simplicial complex whose 2-simplices are all contractible, which implies that $K_ε$ is homotopic to a one-dimensional simplicial complex. Let $H^2(K_ε) = 0$. If $ψ_j$, $ψ_k$, $ψ_l$ are three images that lie in the $ε$-neighborhood of an image $ψ_i$, then at least one of $ψ_j$, $ψ_k$, $ψ_l$ cannot lie in the intersection of $ε$-neighborhoods of the other two. In terms of the graph $G_ε$, $H^2(K_ε) = 0$ implies that $G_ε$ does not contain a complete graph with four vertices.

The isomorphism with $H^2(K_ε)$ also allows us to calculate $\tilde{H}^1(K_ε, SO(2))$ explicitly.

**Theorem 5.2.** $\tilde{H}^1(K_ε, SO(2)) \cong H^2(K_ε) = \mathbb{Z}^b$ where $b = b_2(K_ε)$, the second Betti number of $K_ε$.

**Proof.** The isomorphism is (22). The equality follows from Theorem 3.3, observing that $H_1(K_ε) = 0$ by our construction of $K_ε$ and so $T_1 = 0$. □

This paragraph may be skipped without affecting continuity. We may derive the isomorphism $\tilde{H}^1(K_ε, SO(2)) \cong H^2(K_ε)$ directly without going through the chain of isomorphisms in (22). Consider the exact sequence of groups

$$1 \to \mathbb{Z} \xrightarrow{2π} \mathbb{R} \xrightarrow{\exp i} \mathbb{S}^1 \to 1,$$

where the first map is multiplication by $2π$ and $\exp i(x) := \exp(ix)$. Standard arguments [17, 24, 39] applied to (23) yield a long exact sequence of cohomology groups

$$\cdots \to \tilde{H}^1(K_ε, \mathbb{R}) \to \tilde{H}^1(K_ε, \mathbb{S}^1) \to \tilde{H}^2(K_ε, \mathbb{Z}) \to \tilde{H}^2(K_ε, \mathbb{R}) \to \cdots$$

Both $\tilde{H}^1(K_ε, \mathbb{R})$ and $\tilde{H}^2(K_ε, \mathbb{R})$ are zero by the existence of partition of unity on $K_ε$. So $\tilde{H}^1(K_ε, \mathbb{S}^1) = \tilde{H}^2(K_ε, \mathbb{Z})$. Since $\mathbb{S}^1 = SO(2)$, $\tilde{H}^1(K_ε, \mathbb{S}^1) = \tilde{H}^1(K_ε, SO(2))$. Finally, by Theorem 3.6, we get $\tilde{H}^2(K_ε, \mathbb{Z}) \cong H^2(K_ε, \mathbb{Z}) = H^2(K_ε)$.

5.2. Cohomology as moduli. A benefit of classifying cryo-EM data sets in terms of oriented circle bundles is that these are very well understood classical objects [6, 40]. In what follows, we will refine Theorem 4.2 with explicit descriptions of the oriented circle bundles that arise in the classification of cryo-EM data sets.

Let $b_2(K_ε) = b$. Since $K_ε$ is a finite two-dimensional simplicial complex, this means that it contains $b$ copies of 2-spheres. By (21) and Theorem 5.2, we expect to obtain an oriented circle bundle over $K_ε$ for each $(m_1, \ldots, m_b) \in \mathbb{Z}^b$. An oriented circle bundle over any one-dimensional simplicial complex $K$ must be trivial since $H^2(K) = 0$. Hence any oriented circle bundle over $K_ε$ is uniquely determined by its restriction to the 2-spheres contained in $K_ε$ and our task reduces to oriented circle bundle on $\mathbb{S}^2$, which we will describe explicitly in the following.

We start by identifying the 3-sphere with the group of unit quaternions, i.e.,

$$\mathbb{S}^3 = \{a + bi + cj + dk \in \mathbb{H} : a, b, c, d \in \mathbb{R}, a^2 + b^2 + c^2 + d^2 = 1\},$$

and identify the circle with the group of unit complex numbers, i.e.,

$$\mathbb{S}^1 = \{a + bi \in \mathbb{C} : a, b \in \mathbb{R}, a^2 + b^2 = 1\}.$$

Elements of $\mathbb{S}^1$ may be regarded as unit quaternions with $c = d = 0$ and so $\mathbb{S}^1$ a subgroup of $\mathbb{S}^3$. In particular, $\mathbb{S}^1$ acts on $\mathbb{S}^3$ by quaternion multiplication and we have a group action

$$\varphi : \mathbb{S}^1 \times \mathbb{S}^3 \to \mathbb{S}^3, \quad (x + yi, a + bi + cj + dk) \mapsto xa - yb + (xb + ya)i + (xc - yd)j + (xd + ye)k. \quad (24)$$

As topological spaces we have

$$\mathbb{S}^3/\mathbb{S}^1 \simeq \mathbb{S}^2$$

but note that $\mathbb{S}^1$ is not a normal subgroup of $\mathbb{S}^3$ and so $\mathbb{S}^2$ does not inherit a group structure. Let

$$\pi : \mathbb{S}^3 \to \mathbb{S}^3/\mathbb{S}^1 \simeq \mathbb{S}^2$$

6By a 2-sphere in $K_ε$, we meant the boundary of a 3-simplex, which is homeomorphic to $\mathbb{S}^2$. 
be the natural quotient map.

For \( m \in \mathbb{N} \), let \( C_m \) be the subgroup of \( \mathbb{S}^1 \) generated by \( \exp(2\pi i/m) \), a cyclic group of order \( m \). Each \( C_m \) is also a subgroup of \( \mathbb{S}^3 \) and acts on \( \mathbb{S}^3 \) by quaternion multiplication. Since \( C_m \) is a subgroup of \( \mathbb{S}^1 \), we obtain an induced projection map
\[
\pi_m : \mathbb{S}^3/C_m \to \mathbb{S}^3/\mathbb{S}^1 \simeq \mathbb{S}^2
\]for each \( m \in \mathbb{N} \). The following classic result [40] describes all circle bundles on \( \mathbb{S}^2 \)—there are infinitely many of them, one for each nonnegative integer.

**Proposition 5.3.** For each \( m = 0, 1, 2, \ldots \), there is a circle bundle \( (A_m, \pi_m, \varphi_m) \) with base space \( \mathbb{S}^2 \) where
\[
A_0 = \mathbb{S}^1 \times \mathbb{S}^2, \quad A_m = \mathbb{S}^3/C_m \quad \text{for } m \in \mathbb{N}.
\]
The projection to \( \mathbb{S}^2 \),
\[
\pi_0 : A_0 \to \mathbb{S}^2, \quad \pi_m : A_m \to \mathbb{S}^3/\mathbb{S}^1 \simeq \mathbb{S}^2,
\]is the projection onto the second factor for \( m = 0 \) and the quotient map (25) for \( m \in \mathbb{N} \). The group action \( \varphi_m : \mathbb{S}^1 \times A_m \to A_m \) is the trivial action (any element in \( \mathbb{S}^1 \) acts as identity on \( A_0 \)) for \( m = 0 \) and the action induced by quaternion multiplication \( \varphi \) in (24) for \( m \in \mathbb{N} \). Every circle bundle on \( \mathbb{S}^2 \) is isomorphic to an \( A_m \) for some \( m = 0, 1, 2, \ldots \).

Note that these are \( SO(2) \)-bundles since we regard \( SO(2) = \mathbb{S}^1 \). \( A_0 \) is the trivial circle bundle on \( \mathbb{S}^2 \) and \( A_1 \) is the well-known Hopf fibration. As a manifold, \( A_m = \mathbb{S}^3/C_m \) is orientable for all \( m \in \mathbb{N} \) and so each \( A_m \) comes in two different orientations, which we denote by \( A_m^+ \) and \( A_m^- \). For \( m = 0, 1, 2, \ldots \), we write
\[
B_0 := A_0, \quad B_m := A_m^+, \quad B_m^- := A_m^-.
\]These are the oriented circle bundles on \( \mathbb{S}^2 \).

In the following, we will construct a cryo-EM bundle by gluing oriented circle bundles along the cryo-EM complex \( K_\varepsilon \), attaching a copy of \( B_m \) for some \( m \in \mathbb{Z} \) to each 2-sphere in \( K_\varepsilon \). We then show that these bundles are in one-to-one correspondence with cryo-EM data sets on \( K_\varepsilon \).

Let \( K_\varepsilon \) be a cryo-EM complex with \( b_2(K_\varepsilon) = b \), i.e., \( K_\varepsilon \) contains \( b \) copies of 2-spheres. Label these arbitrarily from \( i = 1, \ldots, b \) and denote them \( S^2_i, \ldots, S^2_b \). For any \((m_1, \ldots, m_b) \in \mathbb{Z}^b \), we may define a principal \( SO(2) \)-bundle \( B_{m_1, \ldots, m_b} \) on \( K_\varepsilon \) as one whose restriction on the \( i \)th 2-sphere in \( K_\varepsilon \) is \( B_{m_i} \), \( i = 1, \ldots, b \), and is trivial elsewhere. We remove all the 2-spheres contained in \( K_\varepsilon \) and let the remaining simplicial complex be
\[
L_\varepsilon := (K_\varepsilon - \bigcup_{i=1}^b S^2_i).
\]As a topological space, \( B_{m_1, \ldots, m_b} \) is the union of \( B_{m_i} \)'s corresponding to each of the 2-spheres and the trivial circle bundle on \( L_\varepsilon \),
\[
B_{m_1, \ldots, m_b} := \left[ \bigcup_{i=1}^b B_{m_i} \right] \cup \left[ L_\varepsilon \times \mathbb{S}^1 \right].
\]

\((B_{m_1, \ldots, m_b}, \pi, \varphi)\) is an oriented circle bundle on \( K_\varepsilon \) with \( \pi \) and \( \varphi \) defined as follows. The projection map \( \pi : B_{m_1, \ldots, m_b} \to K_\varepsilon \) is defined by
\[
\pi(f) = \begin{cases} 
\pi_{m_i}(f), & \text{if } f \in B_{m_i}, \quad i = 1, \ldots, b, \\
\text{pr}_1(f), & \text{if } f \in L_\varepsilon \times \mathbb{S}^1.
\end{cases}
\]

Here \( \text{pr}_1 : L_\varepsilon \times \mathbb{S}^1 \to L_\varepsilon \) is the projection onto the first factor. The group action \( \varphi : SO(2) \times B_{m_1, \ldots, m_b} \to B_{m_1, \ldots, m_b} \) is defined by
\[
\varphi(g, f) = \begin{cases} 
\varphi_{m_i}(g, f), & \text{if } f \in B_{m_i}, \quad i = 1, \ldots, b, \\
f, & \text{if } f \in L_\varepsilon \times \mathbb{S}^1.
\end{cases}
\]
for any \( g \in G \) and \( f \in B_{m_1,\ldots,m_b} \). Furthermore, the intersection of any two simplices in \( K_\varepsilon \) is by our construction either empty or a contractible space and so any bundle is trivial on the intersection.

Every oriented circle bundle on \( K_\varepsilon \) is isomorphic to \( B_{m_1,\ldots,m_b} \) for some \((m_1,\ldots,m_b) \in \mathbb{Z}^b\). We have the following classification theorem for cryo-EM data in terms of \( B_{m_1,\ldots,m_b} \).

**Theorem 5.4** (Bundle Classification of Cryo-EM Data III). Let \( \varepsilon > 0 \) be small enough so that (19) holds and let \( K_\varepsilon \) be the corresponding cryo-EM complex. Let \( b = b_\varepsilon(K_\varepsilon) \). Then each cohomologically equivalent continuous cryo-EM data sets \( z_\varepsilon^c \) on \( K_\varepsilon \) corresponds to an isomorphism classes of oriented circle bundles \( B_{m_1,\ldots,m_b} \) on \( K_\varepsilon \) for \((m_1,\ldots,m_b) \in \mathbb{Z}^b\).

**Proof.** Let \( z_\varepsilon^c = \{g_{ij} \in SO(2) : [i,j] \in K_\varepsilon \} \) and \( z_\varepsilon^{uc} = \{g'_{ij} \in SO(2) : [i,j] \in K_\varepsilon \} \) be cohomologically equivalent cryo-EM data sets on \( K_\varepsilon \), i.e., they are related by (20) for some \( g_i, g_j \in SO(2) \). By Theorem 5.1, \( z_\varepsilon^c \) and \( z_\varepsilon^{uc} \) must correspond to the same oriented circle bundle on \( K_\varepsilon \). \( \Box \)

6. Denoising Cryo-EM Images and Cohomology

Our goal in this section is not to propose any new method for denoising cryo-EM images but to provide some perspectives on existing methods, which work well in practice [37, 38, 34]. We saw in Section 4 that a noiseless discrete cryo-EM data set \( z_\varepsilon^d = \{g_{ij} \in SO(2) : [i,j] \in K_\varepsilon \} \) on \( K_\varepsilon \) satisfies the cocycle condition

\[
g_{ij}g_{jk}g_{ki} = 1, \tag{26}
\]

when \( \varepsilon \) is sufficiently small. In reality, a collection of projected images \( \hat{\Psi} = \{\hat{\psi}_1, \ldots, \hat{\psi}_n\} \) obtained from cryo-EM measurements will be corrupted by noise. As a result, the cryo-EM data set \( \hat{z}_\varepsilon = \{\hat{g}_{ij} \in SO(2) : [i,j] \in K_\varepsilon \} \) obtained from \( \hat{\Psi} \) will not satisfy (26) for any \( \varepsilon > 0 \).

In general cryo-EM images are denoised by class averaging [11]. Noisy images are grouped into classes of similar viewing directions. The within-class average is then taken as an approximation of the noise-free image in that direction. The methods for grouping images into classes [37, 38, 34] are essentially all based on the observation that in the noiseless scenario, the cocycle condition (26) must hold. We will look at a few measures of deviation of cryo-EM data from being a cocycle.

Let \( \hat{z}_\varepsilon^d = \{\hat{g}_{ij} \in SO(2) : [i,j] \in K_\varepsilon \} \) be a cryo-EM data set on \( K_\varepsilon \) computed from a noisy set of projected images \( \hat{\Psi} \). Since \( SO(2) \) can be identified with the circle \( S^1 \), every \( g \in SO(2) \) corresponds to an angle \( \theta \in S^1 \), represented by \( \theta \in [0,2\pi) \). A straightforward measure of deviation of \( z_\varepsilon \) from being a cocycle is given by

\[
\delta(z_\varepsilon^d) = \sum_{i,j,k : [i,j],[i,k],[j,k] \in K_\varepsilon} (\theta_{ij} + \theta_{jk} + \theta_{ki})^2,
\]

where the addition in the parentheses is computed in \( S^1 \), i.e., given by the unique number \( \theta_{ijk} \in [0,2\pi) \) such that

\[
\theta_{ij} + \theta_{jk} + \theta_{ki} = \theta_{ijk} \pmod{2\pi}.
\]

**Lemma 6.1.** \( z_\varepsilon^d \) is a cocycle if and only if \( \delta(z_\varepsilon^d) = 0 \).

Let \( \psi \) be an arbitrary projected image. Then \( \delta(\hat{z}_\varepsilon^d) \) quantifies the obstruction of gluing images in

\[
\hat{z}_\varepsilon^d(\psi) = \{g \cdot \psi : g \in \hat{z}_\varepsilon^d \} = \{\hat{g}_{ij} \cdot \psi : [i,j] \in K_\varepsilon \}
\]
together to get the 3D structure of the molecule. If \( \delta(\hat{z}_\varepsilon^d) \) is small, then \( \hat{z}_\varepsilon^d \) is already close enough to a cocycle and hence every image is good.

On the other hand, if \( \delta(\hat{z}_\varepsilon^d) \) is big, then the following measure allows us to identify subsets of good images, if any. Given an image \( \psi \), whenever \([i,j] \) is an edge of \( K_\varepsilon \) for some \( j \), we want the viewing
direction of \( g_{ij} \cdot \psi \) to be close to that of \( \psi \). This is captured by the quantity \( \rho_i(\hat{\omega}^d) := \delta_i(\hat{\omega}^d)/3\delta(\hat{\omega}^d) \) where

\[
\delta_i(\hat{\omega}^d) = \sum_{j,k : [i,j],[i,k],[j,k] \in K^*} (\theta_{ij} + \theta_{jk} + \theta_{ki})^2, \quad i = 1, \ldots, n.
\]

Clearly, \( \sum_{i=1}^n \rho_i(\hat{\omega}^d) = 1 \). For \( g_{ij} \cdot \psi \) to be a good image, we want \( \rho_i(\hat{\omega}^d) \ll 1 \).

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