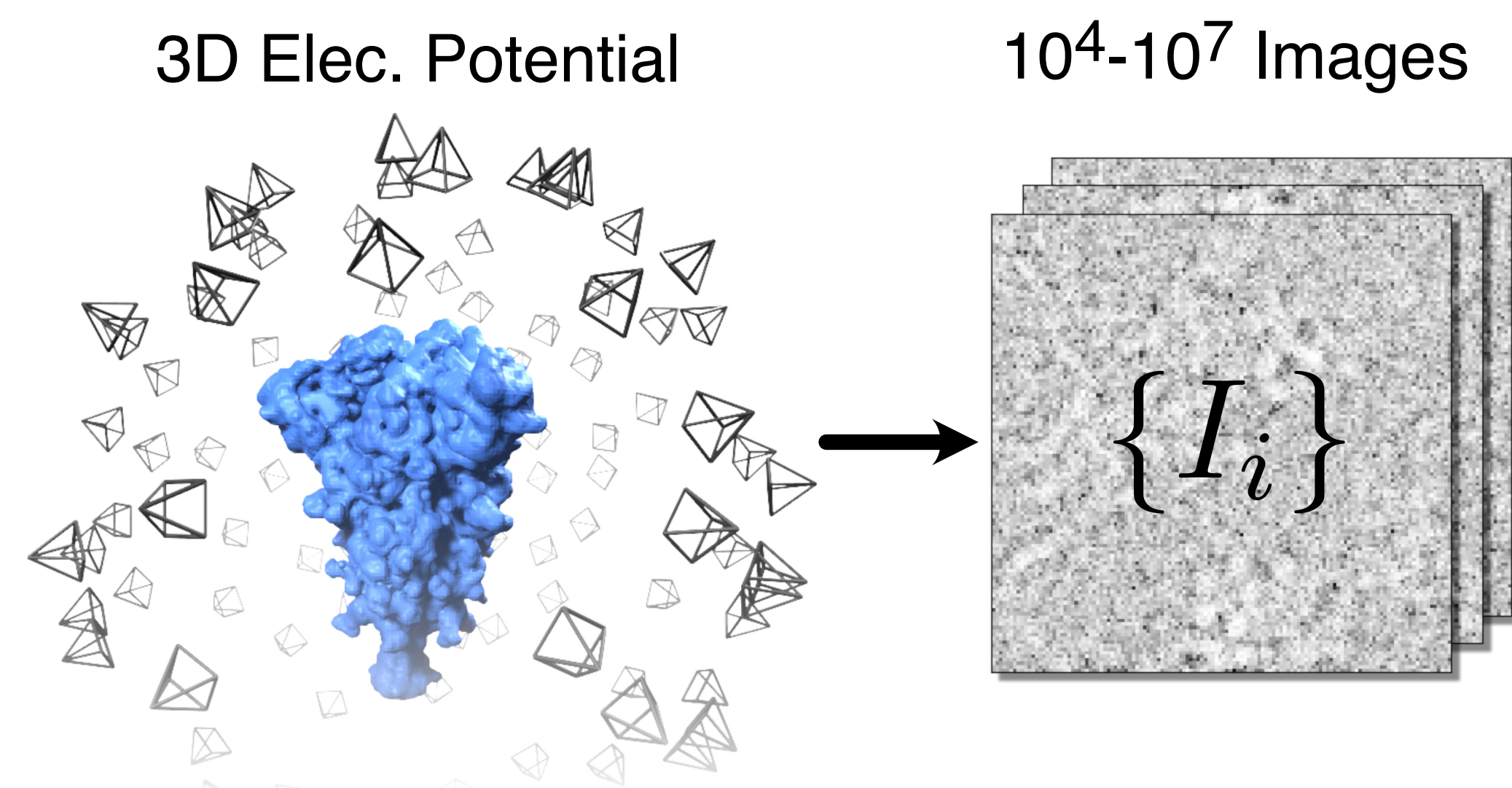


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## Introduction

- The objective of *ab initio* heterogeneous reconstruction is to infer the 3D electron scattering potential of a **deformable** molecule given a set of 2D noisy projections with **unknown poses**.
- Today, this task is only tackled by cryoDRGN2, but the **pose search** step remains its main computational bottleneck.
- We propose cryoFIRE, a **self-supervised** pipeline that **jointly amortizes** the estimation of poses and conformations over the size of the datasets.



**Why do we care?** Understanding the degrees of freedom of proteins and estimating their probability density function on their *conformation manifold* is key to access their functions and dynamics.

## Background

What is cryo-EM? In cryo-electron microscopy, individual copies of macromolecules are flash-frozen and imaged with an electron beam that interacts with the 3D potential of the molecules.

Observations Shared Parameters Latent Variables

$$I = T_t * C * \int_t V_\theta(R \cdot [x, y, t]^T, z) dt + \epsilon$$

$\mathbb{R}^2$  Translation     $\mathbb{R}^{3+d} \rightarrow \mathbb{R}$  Deformable Molecule     $SO(3)$  Rotation     $\mathbb{R}^d$  Shape    Noise

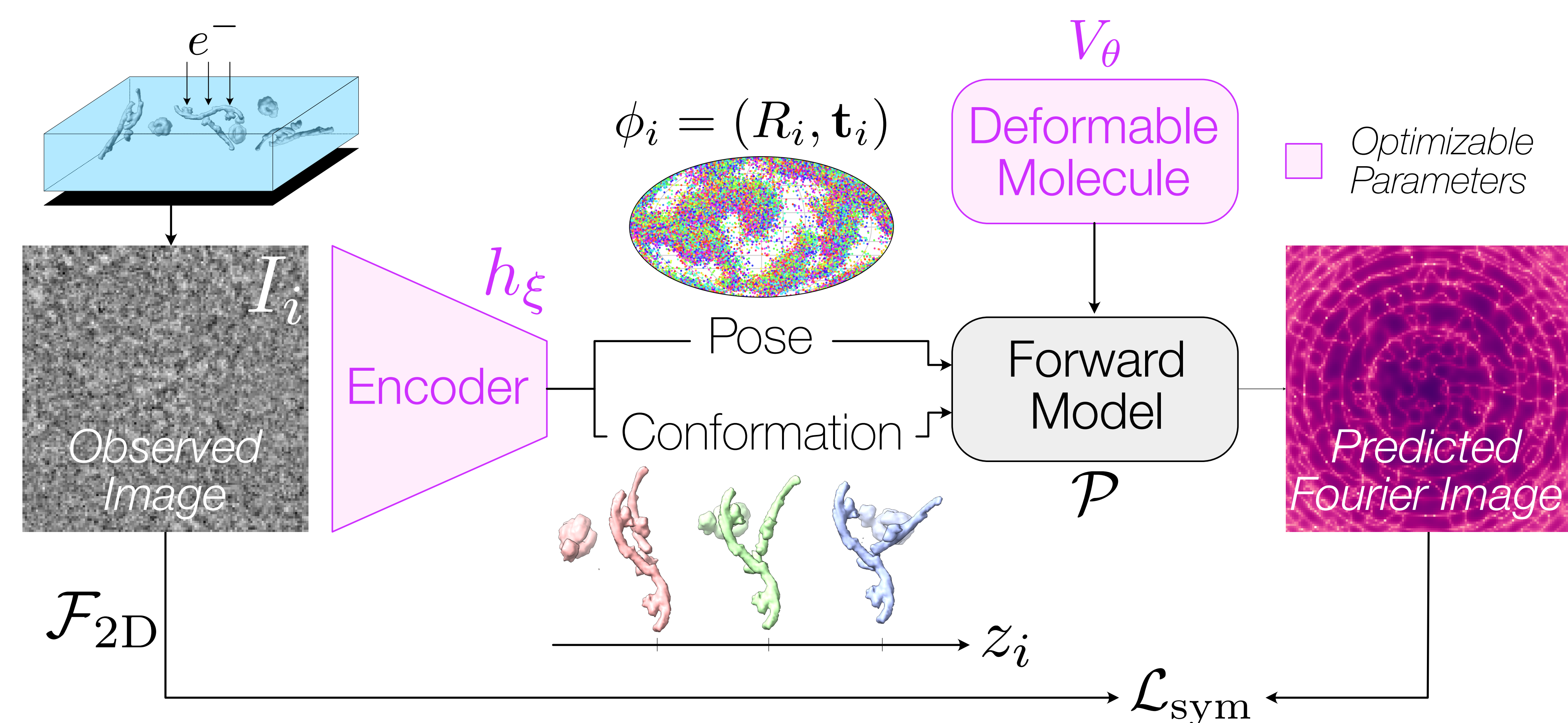
$$\max_{\theta, \{R_i, t_i, z_i\}} \mathbb{P}(\{I_i\} | V_\theta, \{R_i, t_i, z_i\})$$

Joint Amortization

$$\max_{\theta, \xi} \mathbb{P}(\{I_i\} | V_\theta, \{h_\xi(I_i)\})$$

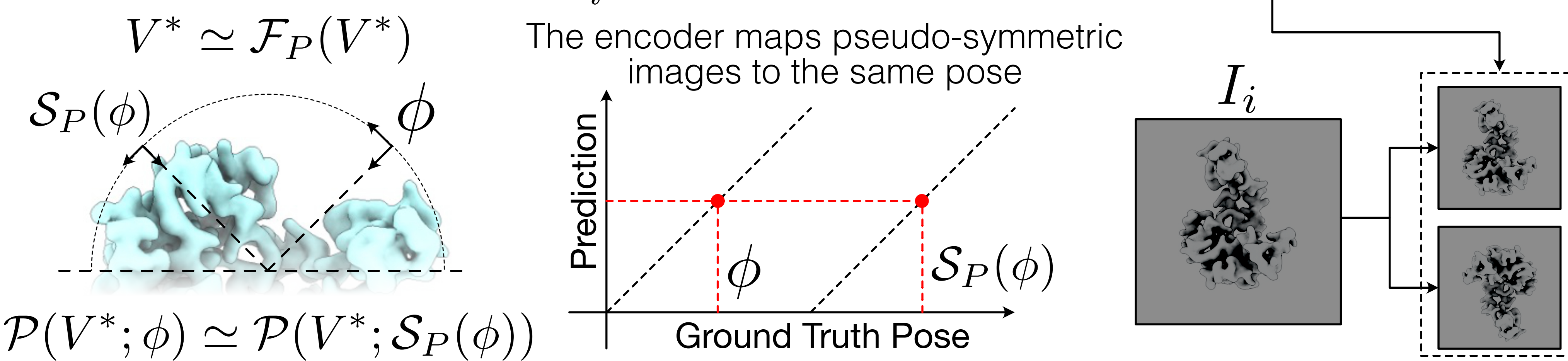
$h$  maps similar images to similar latent variables.

## Method Overview

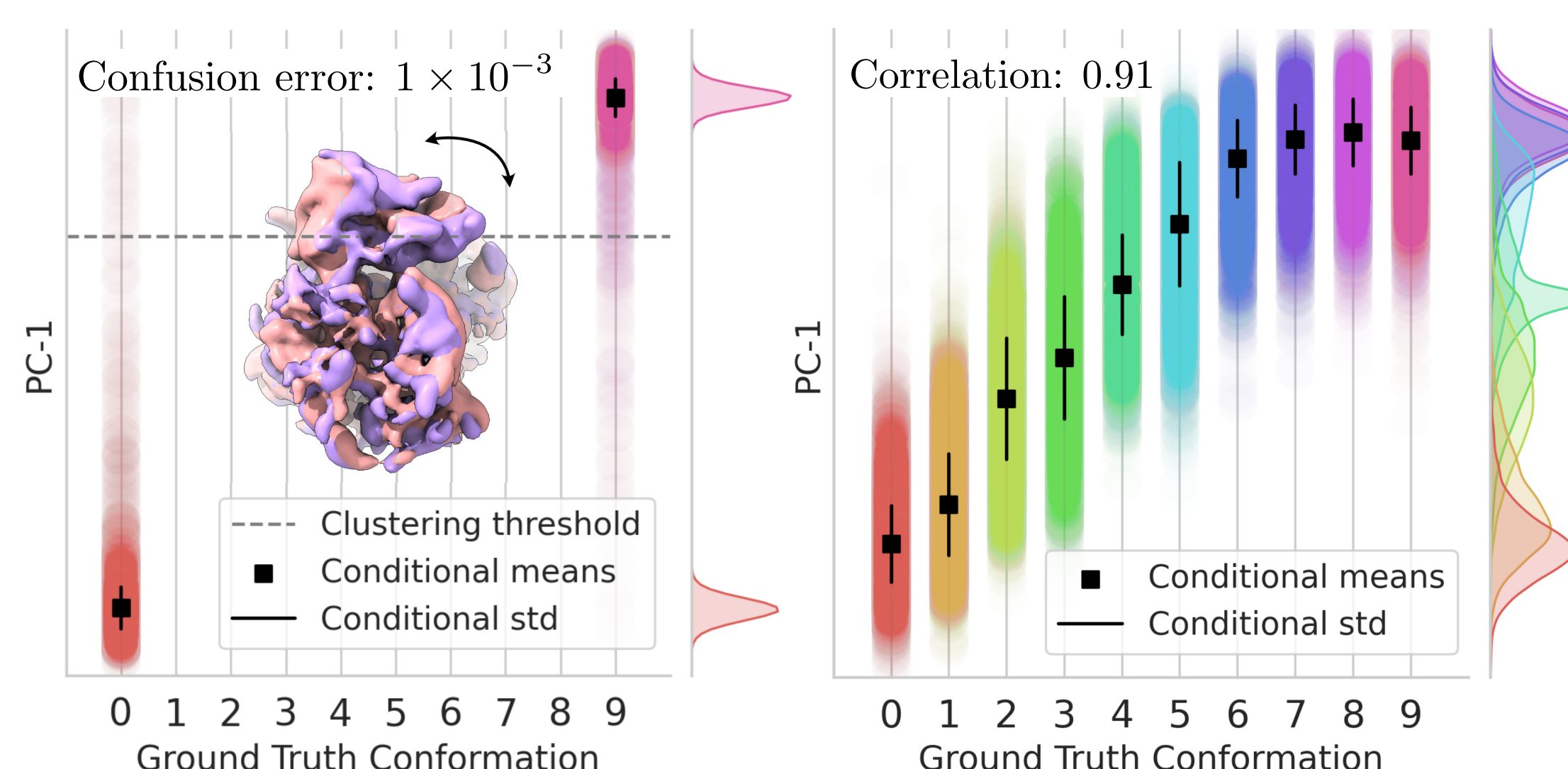


- An **encoder** maps a noisy image to a predicted pose and the conformation.
- A **coordinate-based representation** of the molecule is used in a differentiable simulation of the image formation model to produce a noise-free prediction of the input image.
- We compare, in Fourier space, the predicted image to the observed image with the **symmetric loss** in order to avoid spuriously symmetric states.

$$\mathcal{L}_{\text{sym}}(\xi, \theta) = \sum_i \min\{\|\mathcal{P}(V_\theta; h_\xi(I)) - I\|_2^2, I \in \mathcal{O}(I_i)\}$$



## Performance and Validation

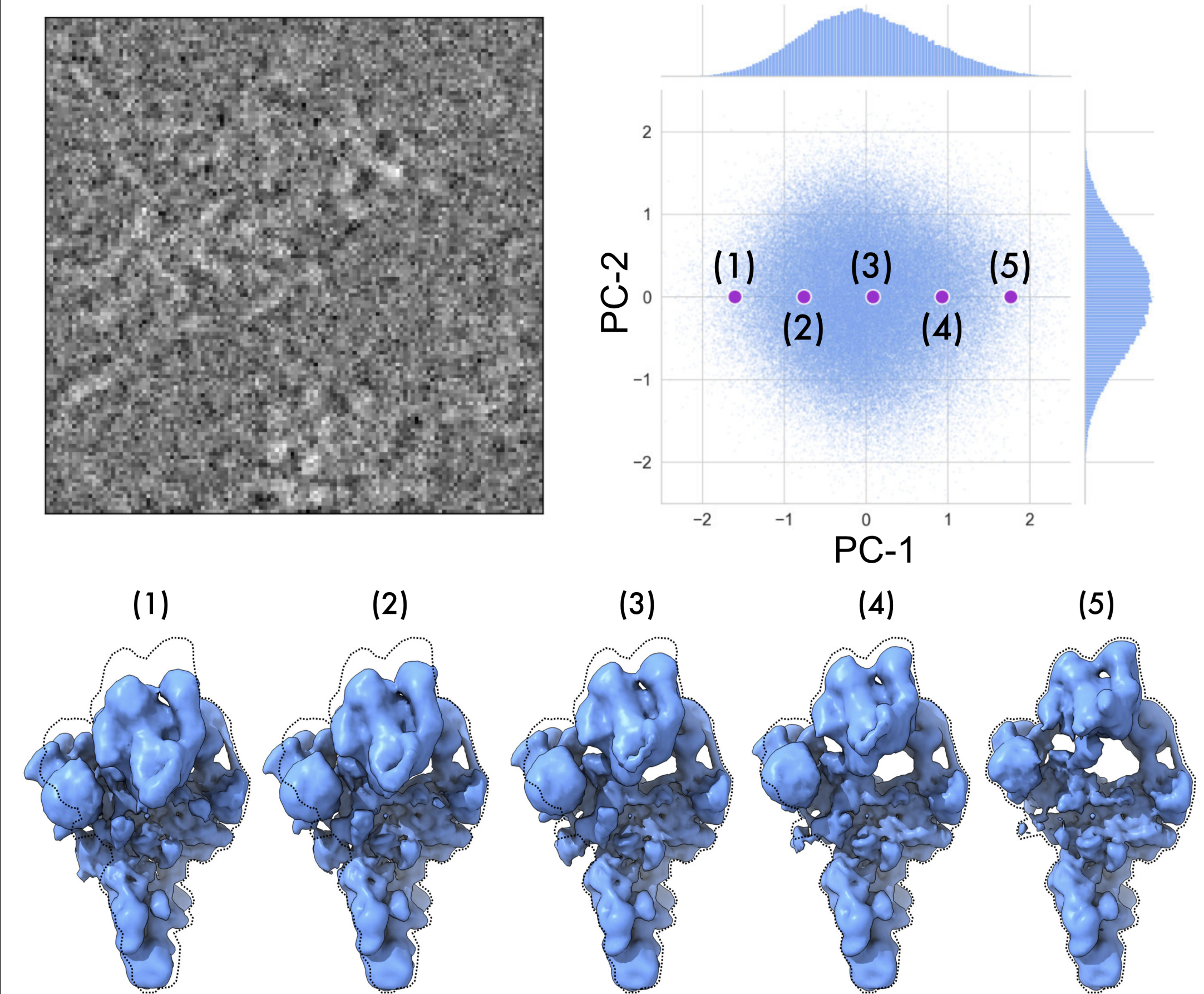


Dataset / Method	Time	Confusion
<b>Large (Train: 5M / Test: 10k)</b>		
cryoDRGN2 (train)	21:37h (1 ep.)	0.002
<b>cryoFIRE (train)</b>	<b>1:55h (1 ep.)</b>	<b>0.0002</b>
cryoDRGN2 (test)	3 min. (1 ep.)	0
<b>cryoFIRE (test)</b>	<b>11 sec. (1 ep.)</b>	<b>0</b>

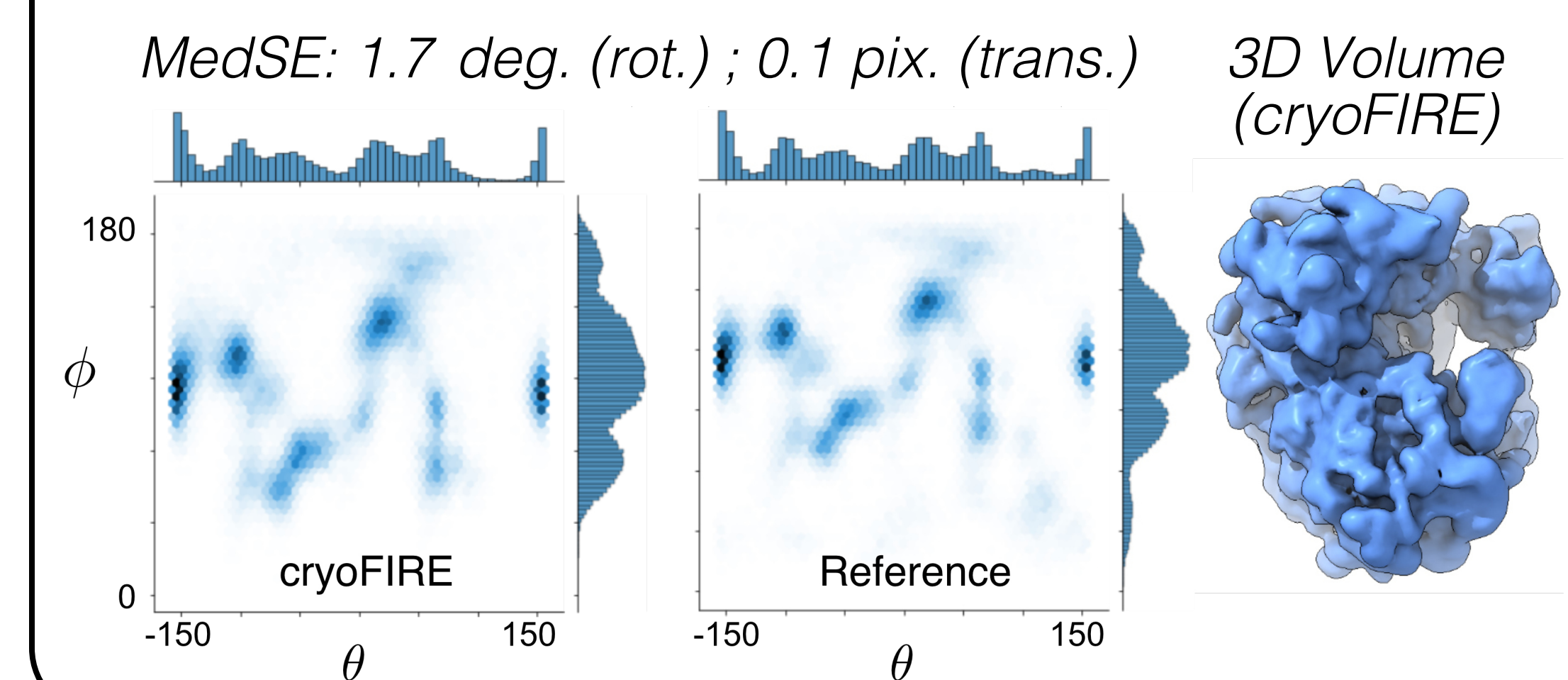
**Speed.** Heterogeneous reconstruction on a large dataset (5M images) is 10x faster than cryoDRGN2, which estimates poses with an exhaustive pose search step.

## Results (real datasets)

**EMPIAR-10180.** *Ab initio* heterogeneous reconstruction of the pre-catalytic spliceosome. Input image, PCA and 3D reconstruction showing the flexing motion of the molecule.



**EMPIAR-10028.** Predicted view directions compared to a published reference of the 80S ribosome and 3D reconstruction.



## Limitations and Scope

- Our parameterization of the conformation manifold is not associated with a physically meaningful metric. Therefore, empirical densities in the conformational space cannot be linked to the energy landscape.
- A change of pose can equivalently be reflected on  $\phi$  or on  $z$ . Future work could explore ways to make  $z$  pose-invariant.
- For more details, please see →