IMPROVED MOTIF-SCAFFOLDING WITH SE(3) FLOW MATCHING

Anonymous authors
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ABSTRACT

Protein design often begins with knowledge of a desired function from a motif which motif-scaffolding aims to construct a functional protein around. Recently, generative models have achieved breakthrough success in designing scaffolds for a diverse range of motifs. However, the generated scaffolds tend to lack structural diversity, which can hinder success in wet-lab validation. In this work, we extend FrameFlow, an $\mathrm{SE}(3)$ flow matching model for protein backbone generation, to perform motif-scaffolding with two complementary approaches. The first is *motif amortization*, in which FrameFlow is trained with the motif as input using a data augmentation strategy. The second is *motif guidance*, which performs scaffolding using an estimate of the conditional score from FrameFlow, and requires no additional training. Our method achieves equivalent success rate than previous state-of-the-art methods, with 2.5 times more structurally diverse scaffolds.

1 INTRODUCTION

A common task in protein design is to create proteins with functional properties conferred through a pre-specified arrangement of residues known as a *motif*. The problem is to design the remainder of the protein, called the *scaffold*, that harbors the motif. Motif-scaffolding is widely used, with applications to vaccine and enzyme design (Procko et al., 2014; Correia et al., 2014; Jiang et al., 2008; Siegel et al., 2010). For this problem, diffusion models have greatly advanced capabilities in designing new scaffolds (Wu et al., 2023; Trippe et al., 2022; Ingraham et al., 2023). While experimental wet-lab validation is the ultimate test for evaluating a scaffold, in this work we focus on improving performance under computational validation of scaffolds following prior works. This involves a designability[1] criteria which has been found to correlate well with wet-lab success (Wang et al., 2021). The current state-of-the-art, RFdiffusion (Watson et al., 2023), fine-tunes a pre-trained RosettaFold (Baek et al., 2023) neural network with $\mathrm{SE}(3)$ diffusion (Yim et al., 2023b) and is able to successfully scaffold the majority of motifs in a recent benchmark.[2] However, RFdiffusion suffers from low scaffold diversity which can hinder chances of a successful design. Moreover, the large model size and pre-training used in RFdiffusion makes it slow to train and difficult to deploy on smaller machines. In this work, we present a lightweight and easy-to-train generative model that provides improved performance.

To achieve diverse scaffolds without relying on pre-training, we adapt an existing $\mathrm{SE}(3)$ flow matching model, FrameFlow (Yim et al., 2023a), for motif-scaffolding. We develop two approaches: (i) *motif amortization*, and (ii) *motif guidance* as illustrated in Fig. 1. Motif amortization was first introduced in RFdiffusion where a *conditional* model is trained with the motif as additional input when generating the scaffold. We show that FrameFlow can be trained in the same way with improved performance. Motif guidance relies on a Bayesian approach, using an *unconditional* FrameFlow model to sample the scaffold residues, while the motif residues are guided at each step to their final desired end positions. An unconditional model in this context is one that generates the full protein backbone without distinguishing between the motif and scaffold. Motif guidance was described in Wu et al. (2023) for $\mathrm{SE}(3)$ diffusion. In this work, we develop the extension to $\mathrm{SE}(3)$ flow matching.

[1] A metric based on using ProteinMPNN (Dauparas et al., 2022) and AlphaFold2 (Jumper et al., 2021) to determine the quality of a protein backbone.

[2] First introduced in RFdiffusion as a benchmark of 24 single-chain motifs successfully solved across prior works published.

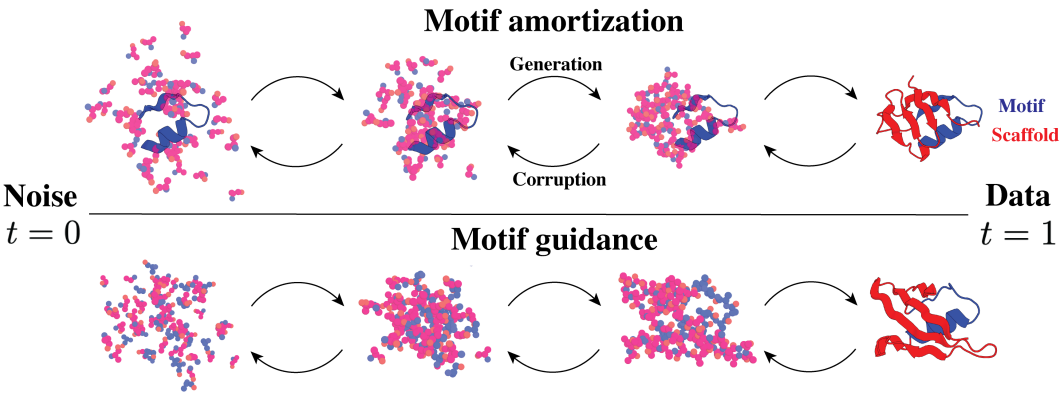

Figure 1: We present two strategies for motif-scaffolding. **Top**: motif amortization trains a flow model to condition on the motif (blue) and generate the scaffold (red). During training, only the scaffold is corrupted with noise. **Bottom**: motif guidance re-purposes a flow model that is trained to generate the full protein for motif-scaffolding. During generation, the motif residues are guided to reconstruct the true motif at $t = 1$ while the flow model will adjust the scaffold trajectory to be consistent with the motif.

The two approaches differ in whether to use an conditional model or to re-purpose an unconditional model for conditional generation. Motif guidance has the advantage that any unconditional model can be used to readily perform motif scaffolding without the need for additional task-specific training. To provide a controlled comparison, we train unconditional and conditional versions of FrameFlow on a dataset of monomers from the Protein Data Bank (PDB) (Berman et al., 2000). Our results provide a clear comparison of the modeling choices made when performing motif-scaffolding with FrameFlow. We find that FrameFlow with both motif amortization and guidance surpasses the performance of RFdiffusion, as measured by the number of unique scaffolds[3] that pass the designability criterion. Our contributions are the following:

- We extend FrameFlow with two fundamentally different approaches for motif-scaffolding: motif amortization and motif guidance. With all other settings kept constant, we show empirical results on motif-scaffolding with each approach.
- Our method can successfully scaffold 20 out of 24 motifs in the motif-scaffolding benchmark which is equivalent to previous state-of-the-art, while achieving 2.5 times more unique, designable scaffolds. Our results demonstrate the importance of measuring quality and diversity.

2 Motif-scaffolding with FrameFlow

We describe our two strategies for performing motif-scaffolding with the FrameFlow model: motif amortization (Sec. 2.1) and motif guidance (Sec. 2.2). Background on FrameFlow is provided in App. C. Recall the full protein backbone is given by $\mathbf{T} = \{T_1, T_2, \ldots, T_N\} \in \mathrm{SE}(3)^N$. The residues can be separated into the motif $\mathbf{T}^M = \{T_{i_1}, \ldots, T_{i_k}\}$ of length k where $\{i_1, \ldots, i_k\} \subset \{1, \ldots, N\}$, and the scaffold $\mathbf{T}^S$ is all the remaining residues, such that $\mathbf{T} = \mathbf{T}^M \cup \mathbf{T}^S$. The task of motif-scaffolding can be framed as sampling from the conditional distribution $p(\mathbf{T}^S|\mathbf{T}^M)$.

2.1 Motif amortization

We train a variant of FrameFlow that additionally takes the motif as input when generating scaffolds (and keeping the motif fixed). Formally, we model a motif-conditioned CNF via the following ODE,

$$\frac{\mathrm{d}}{\mathrm{d}t}\phi_t(\mathbf{T}_0^S|\mathbf{T}^M) = v(\phi_t, t|\mathbf{T}^M), \quad \phi_0(\mathbf{T}_0^S|\mathbf{T}^M) = \mathbf{T}_0^S.$$

The flow ϕ_t transforms a prior density over scaffolds along time, inducing a density $p_t(\cdot|\mathbf{T}^M) = [\phi_t]_* p_0(\cdot|\mathbf{T}^M)$. We use the same prior as in App. C.2: $p_0(\mathbf{T}_0^S|\mathbf{T}^M) = p_0(\mathbf{T}_0^S)$. FrameFlow is trained to predict the conditional vector field $u(\mathbf{T}_t^S, t|\mathbf{T}_1^S, \mathbf{T}^M)$ where $\mathbf{T}_t^S$ is defined by interpolating along the geodesic path, $\mathbf{T}_t^S = \exp_{\mathbf{T}_0^S}\left(t \log_{\mathbf{T}_0^S}(\mathbf{T}_1^S)\right)$. The implication is that u is conditionally

[3]The number of unique scaffolds is defined as the number of structural clusters. See App. G

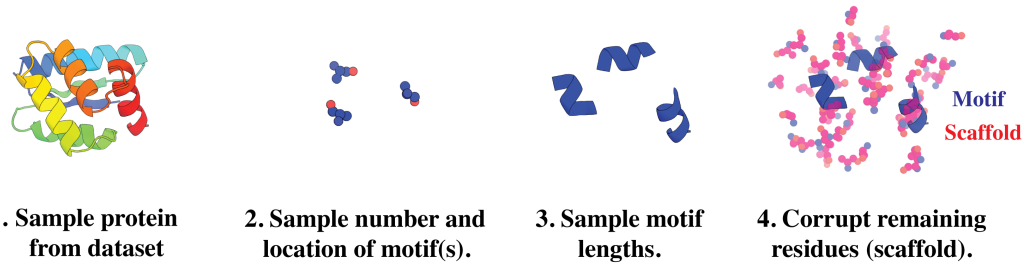

Figure 2: **Motif data augmentation.** Each protein in the dataset does not come with pre-defined motif-scaffold annotations. Instead, we construct plausible motifs at random to simulate sampling from the distribution of motifs and scaffolds.

independent of the motif $\mathbf{T}^M$ given $\mathbf{T}_1^S$. This simplifies our formulation to $u(\mathbf{T}_t^S, t|\mathbf{T}_1^S, \mathbf{T}^M) = u(\mathbf{T}_t^S, t|\mathbf{T}_1^S)$ that is defined in App. C.2. However, when we learn the vector field, the model needs to condition on $\mathbf{T}^M$ since the motif placement $\mathbf{T}^M$ contains information on the true scaffold positions $\mathbf{T}_1^S$. The training loss becomes,

$$\mathbb{E}\left[\left\|\mathbf{u}_{\mathrm{SE}(3)}(\mathbf{T}_t^S, t|\mathbf{T}_1^S) - \hat{\mathbf{v}}_{\mathrm{SE}(3)}(\mathbf{T}_t^S, t|\mathbf{T}^M)\right\|^2_{\mathrm{SE}(3)}\right] \tag{1}$$

where the expectation is taken over $\mathcal{U}(t;[0,1]), p(\mathbf{T}^M), p_1(\mathbf{T}_1^S|\mathbf{T}^M), p_0(\mathbf{T}_0^S)$. The above expectation requires access to the motif and scaffold distributions, $p(\mathbf{T}^M)$ and $p_1(\mathbf{T}_1^S|\mathbf{T}^M)$, during training. While some labels exist for which residues correspond to the functional motif, the vast majority of protein structures in the PDB do not have labels. We instead utilize unlabeled PDB structures to perform data augmentation that allows sampling a wide range of motifs and scaffolds. We discuss this in more details in Sec. 2.1.1. To learn the motif-conditioned vector field $\hat{v}_t$, we use the FrameFlow architecture with a 1D mask as additional input with a 1 at the location of the motif and 0 elsewhere. To maintain $\mathrm{SE}(3)$-equivariance, we zero-center the motif and initial noise sample from $p_0(\mathbf{T}_0^S|\mathbf{T}^M)$.

2.1.1 Data augmentation.

The flow matching loss from Eq. (1) involves sampling from $p(\mathbf{T}^M)$ and $p_1(\mathbf{T}_1^S|\mathbf{T}^M)$, which we do not have access to but can be approximated using unlabeled structures from the PDB. Our pseudo-labeled motifs and scaffolds are generated as follows (also depicted in Fig. 2). First, a protein structure is sampled from the PDB dataset. Second, a random number of residues are selected to be the starting locations of each motif. Third, a random number of additional residues are appended onto each motif thereby extending their lengths. Finally, the remaining residues are treated as the scaffold and corrupted. The motif and scaffold are treated as samples from $p(\mathbf{T}^M)$ and $p_1(\mathbf{T}_1^S|\mathbf{T}^M)$ respectively. Importantly, each protein will be re-used on subsequent epochs where new motifs and scaffolds will be sampled. Our pseudo motif-scaffolds cover a wide range of scenarios that cover multiple motifs of different lengths. We note this strategy was also used in RFdiffusion and bears resemblance to span masking (Hsu et al., 2022). Each step is described in Alg. 1.

2.2 Motif guidance

We now present an alternative Bayesian approach to motif-scaffolding that does not involve learning a motif-conditioned flow model. As such it does not require having access to motifs at training time, but only at sampling time. This can be useful when an unconditional generative flow model is already available at hand and additional training is too costly. The idea behind motif guidance, first described as a special case of TDS (Wu et al., 2023) using diffusion models, is to use the desired motif $\mathbf{T}^M$ to bias the model's generative trajectory such that the motif residues end up in their known positions. The scaffold residues follow a trajectory that create a consistent whole protein backbone, thus achieving motif-scaffolding.

The key insight comes from connecting flow matching to diffusion models to which motif guidance can be applied. The following ODE describes the relationship between the vector field $\hat{\mathbf{v}}$ in flow models – learned by minimizing CFM objective in Eq. (6) – and the Stein score $\nabla \log p_t(\mathbf{T}_t)$,

$$\mathrm{d}\mathbf{T}_t = \hat{\mathbf{v}}(\mathbf{T}_t, t)\mathrm{d}t \left[f(\mathbf{T}_t, t) - \frac{1}{2}g(t)^2 \nabla \log p_t(\mathbf{T}_t)\right] \mathrm{d}t. \tag{2}$$

The gradient is taken with respect to the backbone at time t which we omit for brevity, i.e. $\nabla = \nabla_{\mathbf{T}_t}$. Eq. (2) establishes the connection between the ODE used to sample from flow models and the

probability flow ODE used in sampling from diffusion models (Song et al., 2020) with f and g as the drift and diffusion coefficients.

Our goal is to sample from the conditional $p(\mathbf{T}|\mathbf{T}^M)$ from which we can extract $p(\mathbf{T}^S|\mathbf{T}^M)$. The benefit of Eq. (2) is we can manipulate the score term to achieve this goal. We modify the above to be conditioned on the motif $\mathbf{T}^M$ followed by an application of Bayes rule where $\nabla \log p_t(\mathbf{T}_t|\mathbf{T}^M) = \nabla \log p_t(\mathbf{T}_t) + \nabla \log p_t(\mathbf{T}^M|\mathbf{T}_t)$. We now have

$$\mathrm{d}\mathbf{T}_t = \left[f(\mathbf{T}_t,t) - \frac{1}{2}g(t)^2 \nabla \log p_t(\mathbf{T}_t|\mathbf{T}^M)\right]\mathrm{d}t = \Big[\underbrace{\hat{\mathbf{v}}_{\mathrm{SE}(3)}(\mathbf{T}_t,t)}_{\text{unconditional pred.}} - \tfrac{1}{2}g(t)^2 \underbrace{\nabla \log p_t(\mathbf{T}^M|\mathbf{T}_t)}_{\text{guidance term}}\Big]\mathrm{d}t. \tag{3}$$

We can interpret Eq. (3) as doing unconditional generation by following $\hat{\mathbf{v}}_{\mathrm{SE}(3)}(\mathbf{T},t)$ while $\nabla \log p_t(\mathbf{T}^M|\mathbf{T}_t)$ guides the noised residues so as to be consistent with the true motif. The conditional score $\nabla \log p_t(\mathbf{T}^M|\mathbf{T}_t)$ is unknown, yet it can be approximated by marginalising out $\mathbf{T}_1$ and using the neural network's denoised output (Song et al., 2022; Chung et al., 2022; Wu et al., 2023),

$$\begin{aligned} p_t(\mathbf{T}^M|\mathbf{T}_t) = \int p(\mathbf{T}^M|\mathbf{T}_1)p_{1|t}(\mathbf{T}_1|\mathbf{T}_t)\mathrm{d}\mathbf{T}_1 &\approx \int p(\mathbf{T}^M|\mathbf{T}_1)\delta_{\hat{\mathbf{T}}_1^M(\mathbf{T}_t)}(\mathbf{T}_t)\mathrm{d}\mathbf{T}_1 \\ &= p(\mathbf{T}^M|\hat{\mathbf{T}}_1^M(\mathbf{T}_t)). \end{aligned} \tag{4}$$

We now have the choice to define the likelihood in Eq. (4) to have higher probability the closer it is to the desired motif:

$$p(\mathbf{T}^M|\hat{\mathbf{T}}_1^M(\mathbf{T}_t)) \propto \exp\left(-\|\mathbf{x}^M - \hat{\mathbf{x}}_1^M(\mathbf{T}_t)\|_{\mathbb{R}}^2/\omega_t^2\right)\exp\left(-\|\mathbf{r}^M - \hat{\mathbf{r}}_1^M(\mathbf{T}_t)\|_{\mathrm{SO}(3)}^2/\omega_t^2\right),$$

which is inversely proportional to the distance from the desired motif. Following $\mathrm{SE}(3)$ flow matching, Eq. (3) becomes factorized into the translation and rotation components. Plugging $p(\mathbf{T}^M|\hat{\mathbf{T}}_1^M(\mathbf{T}_t))$ in Eq. (3), we arrive at the following ODE we may sample $p(\mathbf{T}|\mathbf{T}^M)$ from

$$\text{Translations: } \mathrm{d}\mathbf{x}_t = \left[\hat{\mathbf{v}}_{\mathbb{R}}(\mathbf{T}_t,t) + \tfrac{1}{2}g(t)^2\nabla_{\mathbf{x}_t}\|\mathbf{x}^M - \hat{\mathbf{x}}_1^M(\mathbf{T}_t)\|_{\mathbb{R}}^2/\omega_t^2\right]\mathrm{d}t.$$

$$\text{Rotations: } \mathrm{d}\mathbf{r}_t = \left[\hat{\mathbf{v}}_{\mathrm{SO}(3)}(\mathbf{T}_t,t) + \tfrac{1}{2}g(t)^2\nabla_{\mathbf{r}_t}\|\mathbf{r}^M - \hat{\mathbf{r}}_1^M(\mathbf{T}_t)\|_{\mathrm{SO}(3)}^2/\omega_t^2\right]\mathrm{d}t.$$

ω_t is a hyperparameter that controls the magnitude of the guidance towards the desired motif which we set to $\omega_t^2 = (1-t)^2/(t^2 + (1-t)^2)$ as done in Pokle et al. (2023); Song et al. (2021). While different choices of $g(t)$ are possible, Pokle et al. (2023) proposed to use $g(t) = (1-t)/t$ with the motivation that this matches the diffusion coefficient for the diffusion SDE that matches the marginals of the flow ODE. For completeness, we provide the proof for $g(t)$ in App. E. A similar calculation is non-trivial for $\mathrm{SO}(3)$, hence we use the same $g(t)$ as a reasonnable heuristic and observe good performance as done in (Wu et al., 2023).

3 MOTIF-SCAFFOLDING RESULTS

In this section, we report the results of training FrameFlow for motif-scaffolding. App. G describes training, sampling, and metrics. We briefly provide results on unconditional backbone generation in App. H. Additional motif-scaffolding results and analysis is provided in App. I.

Baselines. We use RFdiffusion (Watson et al., 2023) and the Twisted Diffusion Sampler (TDS) (Wu et al., 2023) as baselines. RFdiffusion's performance is reported based on their published samples. TDS reported motif-scaffolding results with arbitrary scaffold lengths that deviated the benchmark. We re-ran TDS with their best settings using $k = 8$ particles on the RFdiffusion benchmark. FrameFlow-amortization refer to our results with motif amortization while FrameFlow-guidance uses motif guidance.

Benchmark. Fig. 3 shows how each method fares against each other in designability and diversity on each motif of the motif-scaffolding benchmark. In general, RFdiffusion tends to get higher designable success rates, but FrameFlow-amortization and FrameFlow-guidance are able to achieve more unique scaffolds per motif. TDS achieves lower designable scaffolds on average, but demonstrates strong performance on a small subset of motifs. Next, Table. 1 provides the number of motifs

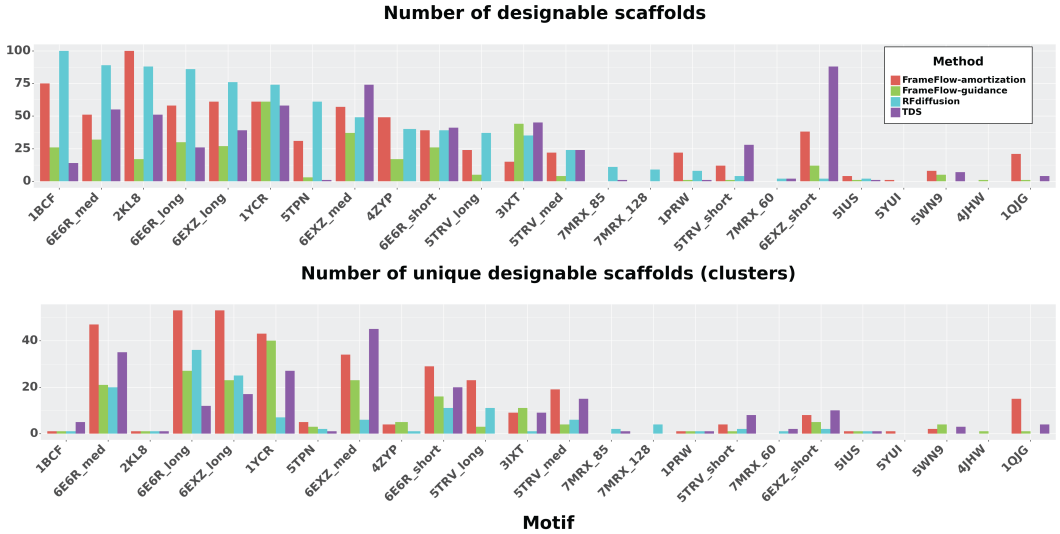

Figure 3: **Motif-scaffolding results.** (Top) Number of designable scaffolds for each motif using each method. While there is no clear trend, RFdiffusion tends to have the highest success rates. (Bottom) Closer inspection into the number of *unique* designable scaffolds (measured as number of clusters) for each method shows a different story where FrameFlow and TDS have more successful scaffolds.

each method solves – which means at least one designable scaffold is sampled – and the number of total designable clusters sampled across all motifs. Here we see each method can solve 19-20 solves motifs, but FrameFlow-amortization can achieve nearly double the number of unique scaffolds (clusters) as RFdiffusion. FrameFlow-amortization outperforms FrameFlow-guidance on diversity. In the last column we give the number of seconds to sample a length 100 protein on a A6000 Nvidia GPU with each method. Both FrameFlow methods are significantly faster than RFdiffusion and TDS. TDS is notably slower since its run time scales with its number of particles. We conclude that FrameFlow-amortization matches RFdiffusion and TDS on the number of solved motifs while achieving much higher diversity.

Table 1: Motif-scaffolding aggregate metrics

Method	**Solved** (↑)	**Div.** (↑)	**Speed** (↓)
FrameFlow-amort.	**20**	**353**	**18s**
FrameFlow-guid.	**20**	192	**18s**
RFdiffusion	**20**	141	50s
TDS	19	217	117s

4 Discussion

In this work, we present two methods building on FrameFlow for tackling motif-scaffolding. These methods can be used with any flow-based model. First, with motif-amortization we adapt the training of FrameFlow to additionally be conditioned on the motif — in effect turning FrameFlow into a conditional generative model. Second, with motif guidance, we use an unconditionally trained FrameFlow for the task of motif-scaffolding though without any additional task-specific training. We empirically evaluated both approaches, FrameFlow-amortization and FrameFlow-guidance, on the motif-scaffolding benchmark from RFdiffusion where we find both methods achieve competitive results with state-of-the-art methods. Moreover, they are able to sample more unique scaffolds and achieve higher diversity. We stress the need to report both success rate and diversity to detect when a model suffers from mode collapse. Lastly, we caveat that all our results and metrics are computational, which may not necessarily transfer to wet-lab success.

Future directions. Our results demonstrate a promising avenue for flow-based models in conditional generation for protein design. We have extended FrameFlow for motif-scaffolding; further extentions include binder, enzyme, and symmetric design — all which RFdiffusion can currently achieve. While motif guidance does not outperform motif amortization, it is possible extending TDS to flow matching could close that gap. Despite our progress, there still remains areas of improvement to achieve success in all 25 motifs in the benchmark.

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A ORGANISATION OF APPENDICES

The appendix is organized as follows. App. B provides background on related work. App. C provides background on FrameFlow. App. D provides details and derivations for FrameFlow (Yim et al., 2023a) that we introduce in App. C.2. App. E provides derivation of motif guidance used in Sec. 2.2. Designability is an important metric in our experients, so we provide a description of it in App. F. Lastly, we include additional motif-scaffolding results and analysis in App. I.

B RELATED WORK

Conditional diffusion and flows. The development of conditional generation methods for diffusion and flow models is an active area of research. Two popular diffusion techniques that have been extended to flow matching are classifier-free guidance (CFG) (Dao et al., 2023; Ho & Salimans, 2022; Zheng et al., 2023) and reconstruction guidance (Pokle et al., 2023; Ho et al., 2022; Song et al., 2022; Chung et al., 2022). Motif guidance is an application of reconstruction guidance for motif-scaffolding. Motif amortization is most related to data-dependent couplings (Albergo et al., 2023), where a flow is learned with conditioning of partial data.

Motif-scaffolding. Wang et al. (2021) first formulated motif-scaffolding using deep learning. SMCDiff (Trippe et al., 2022) was the first proposed diffusion model for motif-scaffolding using Sequential Monte Carlo (SMC). Twisted Diffusion Sampler (TDS) (Wu et al., 2023) later improved upon SMCDiff using reconstruction guidance for each particle in SMC. Our motif guidance method follows from TDS (with one particle) by deriving the equivalent guidance vector field from its conditional score counterpart. RFdiffusion (Watson et al., 2023) fine-tunes a pre-trained neural network with motif-conditioned diffusion training and generation. We train a FrameFlow model with the same motif-conditioned training and generation techniques that we refer to as motif amortization. Compared to RFdiffusion, our method does not rely on expensive pre-training and uses a $3\times$ smaller neural network[4]. Didi et al. (2023) provides a survey of structure-based motif-scaffolding methods while proposing amortized conditioning with Doob's h-transform. Motif amortization is similar to amortized conditioning but uses the unnoised motif as input. EvoDiff (Alamdari et al., 2023) differs in using a sequence-based diffusion model that performs motif-scaffolding with language model-style masked generation but performance falls short of RFdiffusion and TDS. As baselines, we use RFdiffusion and TDS which achieve previous state-of-the-art results.

C BACKGROUND

Flow matching (FM) (Lipman et al., 2023; Albergo et al., 2023) is a simulation-free method for training continuous normalizing flows (CNFs) Chen et al. (2018). CNFs are deep generative models that generates data by integrating an ordinary differential equation (ODE) over a learned vector field. Recently, flow matching has been extended to Riemannian manifolds Chen & Lipman (2023), which we rely on to model protein backbones via the local frame $\mathrm{SE}(3)$ representation. App. C.1 gives an introduction to Riemannian flow matching. App. C.2 then describes how flow matching is applied to protein backbones using $\mathrm{SE}(3)$ flow matching.

C.1 FLOW MATCHING ON RIEMANNIAN MANIFOLDS

On a manifold $\mathcal{M}$, a CNF $\phi_t(\cdot) : \mathcal{M} \to \mathcal{M}$ is defined via an ODE along a time-dependent vector field $v(z, t) : \mathcal{M} \times \mathbb{R} \to \mathcal{T}_z\mathcal{M}$ where $\mathcal{T}_z\mathcal{M}$ is the tangent space of the manifold at $z \in \mathcal{M}$ and time is parameterized by $t \in [0, 1]$:

$$\frac{\mathrm{d}}{\mathrm{d}t}\phi_t(z_0) = v(\phi_t(z_0), t),\ \phi_0(z_0) = z_0. \tag{5}$$

Starting with $z_0 \sim p_0$ from an easy-to-sample prior distribution p_0, simulating samples according to Eq. (5) induces a new distribution referred as the push-forward $p_t = [\phi_t]_* p_0$. One wishes to find a vector field v such that the push-forward $p_{t=1} = [\phi_{t=1}]_* p_0$ (at $t = 1$) matches the data distribution

[4]FrameFlow uses 16.8 million parameters compared to RFdiffusion's 59.8 million.

Algorithm 1 Motif-scaffold data augmentation

Require: Protein backbone $\mathbf{T}$; Min and max motif percent γ_{min}, γ_{max}.

$s \sim \text{Uniform}\{\lfloor N \cdot \gamma_{\text{min}} \rfloor, \ldots, \lfloor N \cdot \gamma_{\text{max}} \rfloor\}$ ▷ Sample maximum motif size.
$m \sim \text{Uniform}\{1, \ldots, s\}$ ▷ Sample maximum number of motifs.
$\mathbf{T}^M \leftarrow \emptyset$
for $i \in \{1, \ldots, m\}$ **do**
  $j \sim \text{Uniform}\{1, \ldots, N\} \setminus \mathbf{T}^M$ ▷ Sample location for each motif
  $\ell \sim \text{Uniform}\{1, \ldots, s - m + i - |\mathbf{T}^M|\}$ ▷ Sample length of each motif.
  $\mathbf{T}^M \leftarrow \mathbf{T}^M \cup \{T_j, \ldots, T_{\text{min}(j+\ell, N)}\}$ ▷ Append to existing motif.
end for
$\mathbf{T}^S \leftarrow \{T_1, \ldots, T_N\} \setminus \mathbf{T}^M$ ▷ Assign rest of residues as the scaffold
return $\mathbf{T}^M, \mathbf{T}^S$

p_1. Such a vector field v is in general not available in closed-form, but can be learned by regressing conditional vector fields $u(z_t, t|z_1) = \frac{\mathrm{d}}{\mathrm{d}t} z_t$ where $z_t = \phi_t(z_0|z_1)$ interpolates between endpoints $z_0 \sim p_0$ and $z_1 \sim p_1$. A natural choice for z_t is the geodesic path: $z_t = \exp_{z_0}\left(t \log_{z_0}(z_1)\right)$, where $\exp_{z_0}$ and $\log_{z_0}$ are the exponential and logarithmic maps at the point z_0. The conditional vector field takes the following form: $u(z_t, t|z_1) = \log_{z_t}(z_1)/(1-t)$. The key insight of conditional[5] flow matching (CFM) (Lipman et al., 2023) is that training a neural network $\hat{v}$ to regress the conditional vector field u is equivalent to learning the unconditional vector field v. This corresponds to minimizing

$$\mathcal{L} = \mathbb{E}_{\mathcal{U}(t;[0,1]), p_1(z_1), p_0(z_0)} \left[\|u(z_t, t|z_1) - \hat{v}(z_t, t)\|_g^2 \right]$$

where $\mathcal{U}(t;[0,1])$ is the uniform distribution for $t \in [0,1]$ and $\|\cdot\|_g^2$ is the norm induced by the Riemannian metric $g : \mathcal{TM} \times \mathcal{TM} \to \mathbb{R}$. Samples can then be generated by integrating the ODE in Eq. (5) with Euler steps using the learned vector field $\hat{v}$ in place of v.

C.2 Generative modeling on protein backbones

The atom positions of each residue in a protein backbone can be parameterized by an element $T \in \text{SE}(3)$ of the special Euclidean group $\text{SE}(3)$ (Jumper et al., 2021; Yim et al., 2023b). We refer to $T = (r, x)$ as a (local) frame consisting of a rotation $r \in \text{SO}(3)$ and translation vector $x \in \mathbb{R}^3$. The protein backbone is made of N residues, meaning it can be parameterized by N frames denoted as $\mathbf{T} = [T^{(1)}, \ldots, T^{(N)}] \in \text{SE}(3)^N$. We use bold face to refer to vectors of all the residues, superscripts to refer to residue indices, and subscripts refer to time. Details of the $\text{SE}(3)^N$ backbone parameterization can be found in App. D.1.

We use $\text{SE}(3)$ flow matching to parameterize a generative model over the $\text{SE}(3)^N$ representation of protein backbones. The application of Riemannian flow matching to $\text{SE}(3)$ was previously developed in Yim et al. (2023a); Bose et al. (2023). Endowing $\text{SE}(3)$ with the product left-invariant metric, the $\text{SE}(3)$ manifold effectively behaves as the product manifold $\text{SE}(3) = \text{SO}(3) \times \mathbb{R}^3$ (App. D.3 of Yim et al. (2023b)). The vector field over $\text{SE}(3)$ can then be decomposed as $v^{(n)}_{\text{SE}(3)}(\cdot, t) = (v^{(n)}_{\mathbb{R}}(\cdot, t), v^{(n)}_{\text{SO}(3)}(\cdot, t))$. We parameterize each vector field as

$$\hat{v}^{(n)}_{\mathbb{R}}(\mathbf{T}_t, t) = \frac{\hat{x}^{(n)}_1(\mathbf{T}_t) - x^{(n)}_t}{1-t},$$
$$\hat{v}^{(n)}_{\text{SO}(3)}(\mathbf{T}_t, t) = \frac{\log_{r^{(n)}_t}(\hat{r}^{(n)}_1(\mathbf{T}_t))}{1-t}.$$

[5]Unfortunately the meaning of "conditional" is overloaded. The conditionals will be clear from the context.

The outputs of the neural network are *denoised* predictions $\hat{x}_1^{(n)}$ and $\hat{r}_1^{(n)}$ which are used to calculate the vector fields. The loss becomes,

$$\begin{aligned}\mathcal{L}_{\mathrm{SE}(3)} &= \mathbb{E}\left[\left\|\mathbf{u}_{\mathrm{SE}(3)}(\mathbf{T}_t, t|\mathbf{T}_1) - \hat{\mathbf{v}}_{\mathrm{SE}(3)}(\mathbf{T}_t, t)\right\|^2_{\mathrm{SE}(3)}\right] \\ &= \mathbb{E}\Big[\left\|\mathbf{u}_{\mathbb{R}}(\mathbf{x}_t, t|\mathbf{x}_1) - \hat{\mathbf{v}}_{\mathbb{R}}(\mathbf{T}_t, t)\right\|^2_{\mathbb{R}} + \\ &\qquad \left\|\mathbf{u}_{\mathrm{SO}(3)}(\mathbf{r}_t, t|\mathbf{r}_1) - \hat{\mathbf{v}}_{\mathrm{SO}(3)}(\mathbf{T}_t, t)\right\|^2_{\mathrm{SO}(3)}\Big]\end{aligned} \tag{6}$$

where the expectation is taken over $\mathcal{U}(t;[0,1])$, $p_1(\mathbf{T}_1)$, $p_0(\mathbf{T}_0)$. We have used bold-face for collections of elements, i.e. $\hat{\mathbf{v}}(\cdot) = [\hat{v}^{(1)}(\cdot), \ldots, \hat{v}^{(N)}(\cdot)]$. Our prior is chosen as $p_0(\mathbf{T}_0) = \mathcal{U}(\mathrm{SO}(3))^N \otimes \overline{\mathcal{N}}(0, I_3)^N$, where $\mathcal{U}(\mathrm{SO}(3))$ is the uniform distribution over $\mathrm{SO}(3)$ and $\overline{\mathcal{N}}(0, I_3)$ is the isotropic Gaussian where samples are centered to the origin.

Details of $\mathrm{SE}(3)$ flow matching closely follow FrameFlow (Yim et al., 2023a) where additional auxiliary losses are used. To learn the vector field, we use the neural network architecture from FrameDiff (Yim et al., 2023b) that is comprised of layers that use Invariant Point Attention (IPA) (Jumper et al., 2021) and Transformer blocks (Vaswani et al., 2017). FrameFlow details are provided in App. D.2.

D FRAMEFLOW DETAILS

D.1 BACKBONE SE(3) REPRESENTATION

A protein can be described by its sequence of residues, each of which takes on a discrete value from a vocabulary of amino acids, as well as the 3D structure based on the positions of atoms within each residue. The 3D structure in each residue can be separated into the backbone and side-chain atoms with the composition of backbone atoms being constant across all residues while the side-chain atoms vary depending on the amino acid assignment. For this reason, FrameFlow and previous $\mathrm{SE}(3)$ diffusion models (Watson et al., 2023; Yim et al., 2023b) only model the backbone atoms with the amino acids assumed to be unknown. A second model is typically used to design the amino acids after the backbone is generated. Each residue's backbone atoms follows a repeated arrangement with limited degrees of freedom due to the rigidity of the covalent bonds. AlphaFold2 (AF2) (Jumper et al., 2021) proposed a $\mathrm{SE}(3)$ parameterization of the backbone atoms that we show in Fig. 4. AF2 uses a mapping of four backbone atoms to a single translation and rotation that reduces the degrees of freedom in the modeling. It is this $\mathrm{SE}(3)$ representation we use when modeling protein backbones. We refer to Appendix I of Yim et al. (2023b) for algorithmic details of mapping between elements of $\mathrm{SE}(3)$ and backbone atoms.

D.2 SE(3) FLOW MATCHING IMPLEMENTATION

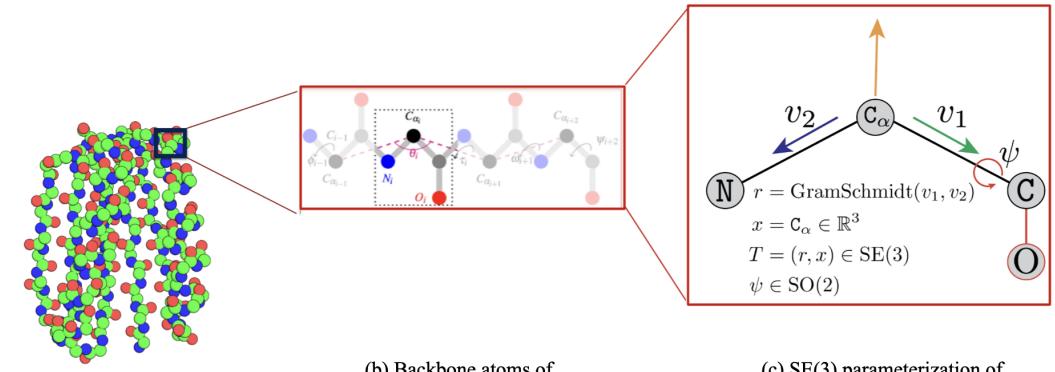
This section provides implementation details for $\mathrm{SE}(3)$ flow matching and FrameFlow. As stated in App. C.1, $\mathrm{SE}(3)^N$ can be characterized as the product manifold $\mathrm{SE}(3)^N = \mathbb{R}^N \times \mathrm{SO}(3)^N$. It follows that flow matching on $\mathrm{SE}(3)^N$ is equivalent to flow matching on $\mathbb{R}^{3N}$ and $\mathrm{SO}(3)^N$. We will parameterize backbones with N residues $\mathbf{T} = (\mathbf{x}, \mathbf{r}) \in \mathrm{SE}(3)^N$ by translations $\mathbf{x} \in \mathbb{R}^{3N}$ and rotations $\mathbf{r} \in \mathrm{SO}(3)^N$. As a reminder, we use bold face for vectors of all the residue: $\mathbf{T} = [T^{(1)}, \ldots, T^{(N)}]$, $\mathbf{x} = [x^{(1)}, \ldots, x^{(N)}]$, $\mathbf{r} = [r^{(1)}, \ldots, r^{(N)}]$.

Riemannian flow matching (App. C.1) proceeds by defining the conditional flows,

$$x_t^{(n)} = (1-t)x_0^{(n)} + t x_1^{(n)}, \qquad r_t^{(n)} = \exp_{r_0^{(n)}}\left(t \log_{r_0^{(n)}}(r_1^{(n)})\right), \tag{7}$$

for each residue $n \in \{1, \ldots, N\}$. As priors we use $x_0^{(n)} \sim \overline{\mathcal{N}}(0, I_3)$ and $r_0^{(n)} \sim \mathcal{U}(\mathrm{SO}(3))$. $\overline{\mathcal{N}}(0, I_3)$ is the isotropic Gaussian in 3D with centering where each sample is centered to have zero mean – this is important for equivariance later on. $\mathcal{U}(\mathrm{SO}(3))$ is the uniform distribution over $\mathrm{SO}(3)$. The end points $x_1^{(n)}$ and $r_1^{(n)}$ are samples from the data distribution p_1.

Eq. (7) uses the geodesic path with linear interpolation; however, alternative conditional flows can be used (Chen & Lipman, 2023). A special property of $\mathrm{SO}(3)$ is that $\exp_{r_0}$ and $\log_{r_0}$ can be computed



(a) Protein backbone atoms

(b) Backbone atoms of a single residue

(c) SE(3) parameterization of backbone atoms of a single residue

Figure 4: Backbone parameterization with SE(3). (a) Shows the full protein backbone atomic structure without side-chains. (b) Zooms in the backbone atoms of a single residue. Note the repeated arrangements of backbone atoms in each residue. (c) The transformation of turning each set of four backbone atoms into an element of SE(3).

in closed form using the well known Rodrigues' formula. The corresponding conditional vector fields are

$$u_{\mathbb{R}}^{(n)}(x_t^{(n)}, t | x_1^{(n)}) = \frac{x_1^{(n)} - x_t^{(n)}}{1-t}, \qquad u_{\text{SO}(3)}^{(n)}(r_t^{(n)}, t | r_1^{(n)}) = \frac{\log_{r_t^{(n)}}(r_1^{(n)})}{1-t}. \tag{8}$$

We train neural networks to regress the conditional vector fields through the following parameterization,

$$\hat{v}_{\mathbb{R}}^{(n)}(\mathbf{T}_t, t) = \frac{\hat{x}_1^{(n)}(\mathbf{T}_t) - x_t^{(n)}}{1-t}, \quad \hat{v}_{\text{SO}(3)}^{(n)}(\mathbf{T}_t, t) = \frac{\log_{r_t^{(n)}}(\hat{r}_1^{(n)}(\mathbf{T}_t))}{1-t},$$

where the neural network outputs the *denoised* predictions $\hat{x}_1^{(n)}$ and $\hat{r}_1^{(n)}$ while using the noised backbone $\mathbf{T}_t$ as input. We now modify the loss from Eq. (6) with practical details from FrameFlow,

$$\mathcal{L}_{\text{SE}(3)} = \mathbb{E}_{\mathcal{U}(t;0,1), p_1(\mathbf{T}_1), p_0(\mathbf{T}_0)} \left[\mathcal{L}_{\mathbb{R}}(\mathbf{T}_t, \mathbf{T}_1, t) + 2\mathcal{L}_{\text{SO}(3)}(\mathbf{T}_t, \mathbf{T}_1, t) + \mathbb{1}(t > 0.5)\mathcal{L}_{\text{aux}}(\mathbf{T}_t, \mathbf{T}_1, t) \right]$$

$$\mathcal{L}_{\mathbb{R}}(\mathbf{T}_t, \mathbf{T}_1, t) = \|\mathbf{u}_{\mathbb{R}}(\mathbf{x}_t | \mathbf{x}_1, t) - \hat{\mathbf{v}}_{\mathbb{R}}(\mathbf{T}_t, t)\|_{\mathbb{R}}^2 = \frac{\|\mathbf{x}_1 - \hat{\mathbf{x}}_1\|_{\mathbb{R}}^2}{(1 - \min(t, 0.9))^2} \tag{9}$$

$$\mathcal{L}_{\text{SO}(3)}(\mathbf{T}_t, \mathbf{T}_1, t) = \left\|\mathbf{u}_{\text{SO}(3)}(\mathbf{r}_t | \mathbf{r}_1, t) - \hat{\mathbf{v}}_{\text{SO}(3)}(\mathbf{T}_t, t)\right\|_{\text{SO}(3)}^2 = \frac{\left\|\log_{\mathbf{r}_t^{(n)}}(\mathbf{r}_1^{(n)}) - \log_{\mathbf{r}_t^{(n)}}(\hat{\mathbf{r}}_1^{(n)}(\mathbf{T}_t))\right\|_{\text{SO}(3)}^2}{(1 - \min(t, 0.9))^2}. \tag{10}$$

We up weight the SO(3) loss $\mathcal{L}_{\text{SO}(3)}$ such that it is on a similar scale as the translation loss $\mathcal{L}_{\mathbb{R}}$. Eq. (9) is simplified to be a loss directly on the denoised predictions. Both Eq. (9) and Eq. (10) have modified denominators $(1 - \min(t, 0.9))^{-2}$ instead of $(1-t)^{-1}$ to avoid the loss blowing up near $t \approx 1$. In practice, we sample t uniformly from $\mathcal{U}[\epsilon, 1]$ for small ϵ. Lastly, $\mathcal{L}_{\text{aux}}$ is taken from section 4.2 in Yim et al. (2023b) where they apply a RMSD loss over the full backbone atom positions and pairwise distances. We found using $\mathcal{L}_{\text{aux}}$ for all $t > 0.5$ to be helpful. The remainder of this section goes over additional details in FrameFlow.

Alternative SO(3) prior. Rather than using the $\mathcal{U}(\text{SO}(3))$ prior during training, we find using the IGSO3($\sigma = 1.5$) prior (Nikolayev & Savyolov, 1970) used in FrameDiff to result in improved performance. The choice of $\sigma = 1.5$ will shift the r_0 samples away from π where near degenerate solutions can arise in the geodesic. During sampling, we still use the $\mathcal{U}(\text{SO}(3))$ prior.

Pre-alignment. Following Klein et al. (2023) and Shaul et al. (2023), we pre-align samples from the prior and the data by using the Kabsch algorithm to align the noise with the data to remove any

global rotation that results in a increased kinetic energy of the ODE. Specifically, for translation noise $\mathbf{x}_0 \sim \mathcal{N}(0, \mathrm{I}_3)^N$ and data $\mathbf{x}_1 \sim p_1$ where $\mathbf{x}_0, \mathbf{x}_1 \in \mathbb{R}^{3\times N}$ we solve $r^* =_{r\in \mathrm{SO}(3)} \|r\mathbf{x}_0 - \mathbf{x}_1\|^2_{\mathbb{R}}$ and use the *aligned* noise $r^*\mathbf{x}_0$ during training. We found pre-aligment to aid in training efficiency.

Symmetries. We perform all modelling within the zero center of mass (CoM) subspace of $\mathbb{R}^{N\times 3}$ as in Yim et al. (2023b). This entails simply subtracting the CoM from the prior sample $\mathbf{x}_0$ and all datapoints $\mathbf{x}_1$. As $\mathbf{x}_t$ is a linear interpolation between the noise sample and data, $\mathbf{x}_t$ will have 0 CoM also. This guarantees that the distribution of sampled frames that the model generates is $\mathrm{SE}(3)$-invariant. To see this, note that the prior distribution is $\mathrm{SE}(3)$-invariant and the learned vector field $\mathbf{v}_{\mathrm{SE}(3)}$ is equivariant because we use an $\mathrm{SE}(3)$-equivariant architecture. Hence by Köhler et al. (2020), the push-forward of the prior under the flow is invariant.

SO(3) inference scheduler. The conditional flow in Eq. (7) uses a constant linear interpolation along the geodesic path where the distance of the current point x to the endpoint x_1 is given by a pre-metric $d_g : \mathcal{M} \times \mathcal{M} \to \mathbb{R}$ induced by the Riemannian metric g on the manifold. To see this, we first recall the general form of the conditional vector field with $x, x_1 \in \mathcal{M}$ is given as follows (Chen & Lipman, 2023),

$$\begin{aligned} u_t(x|x_1) &= \frac{\mathrm{d}\log\kappa(t)}{\mathrm{d}t} d(x, x_1) \frac{\nabla d(x, x_1)}{\|\nabla d(x, x_1)\|^2} \\ &= \frac{\mathrm{d}\log\kappa(t)}{\mathrm{d}t} \frac{\nabla d(x, x_1)^2}{2\|\nabla d(x, x_1)\|^2} \\ &= \frac{\mathrm{d}\log\kappa(t)}{\mathrm{d}t} \frac{-\log_x(x_1)}{\|\nabla d(x, x_1)\|^2} \\ &= \frac{-\mathrm{d}\log\kappa(t)}{\mathrm{d}t} \log_x(x_1), \end{aligned}$$

with $\kappa(t)$ a monotonically decreasing differentiable function satisfying $\kappa(0) = 1$ and $\kappa(1) = 0$, referred as the *interpolation rate* [6]. Then plugging in a the linear schedule $\kappa(t) = 1 - t$, we recover Eq. (8)

$$u_t(x|x_1) = \frac{-\mathrm{d}\log\kappa(t)}{\mathrm{d}t} \log_x(x_1) = \frac{1}{1-t} \log_x(x_1).$$

However, we found this interpolation rate to perform poorly for $\mathrm{SO}(3)$ for inference time. Instead, we utilize an exponential scheduler $\kappa(t) = e^{-ct}$ for some constant c. The intuition being that for high c, the rotations accelerate towards the data faster than the translations which evolve according to the linear schedule. The $\mathrm{SO}(3)$ conditional flow in Eq. (7) and vector field in Eq. (8) become the following with the exponential schedule,

$$\begin{aligned} r_t &= \exp_{r_0}\left(\left(1 - e^{-ct}\right)\log_{r_0}(r_1)\right) \\ v_r^{(n)} &= c\log_{r_t^{(n)}}\left(\hat{r}_1^{(n)}\right). \end{aligned} \tag{11}$$

We find $c = 10$ or 5 to work well and use $c = 10$ in our experiments. Interestingly, we found the best performance when $\kappa(t) = 1 - t$ was used for $\mathrm{SO}(3)$ during training while $\kappa(t) = e^{-ct}$ is used during inference. We found using $\kappa(t) = e^{-ct}$ during training made training too easy with little learning happening.

The vector field in Eq. (11) matches the vector field in FoldFlow when inference *annealing* is performed (Bose et al., 2023). However, their choice of scaling was attributed to normalizing the predicted vector field rather than the schedule. Indeed they proposed to linearly scale up the learnt vector field via $\lambda(t) = (1 - t)c$ at sampling time, i.e. to simulate the following ODE:

$$\mathrm{d}r_t = \lambda(t)v(r_t, t)\mathrm{d}t.$$

However, as hinted at earlier, this is equivalent to using at sampling time a different vector field $\tilde{v}(r_t, t)$—induced by an *exponential* schedule $\tilde{\kappa}(t) = e^{-ct}$—instead of the *linear* schedule $\kappa(t) =$

[6] $\kappa(t)$ acts as a scheduler that determines the rate at which $d(\cdot|x_1)$ decreases, since we have that ϕ_t decreases $d(\cdot, x_1)$ according to $d(\phi_t(x_0|x_1), x_1) = \kappa(t)d(x_0, x_1)$(Chen & Lipman, 2023).

$1-t$ (that the neural network $\hat{r}_1^\theta$ was trained with). Indeed we have

$$\begin{aligned}\tilde{v}(r_t,t) &= -\partial_t \log \tilde{\kappa}(t) \log_{r_t}(\hat{r}_1) = -\frac{-\partial_t \log \tilde{\kappa}(t)}{-\partial_t \log \kappa(t)} \partial_t \log \kappa(t) \log_{r_t}(\hat{r}_1) \\ &= -c(1-t)\partial_t \log \kappa(t) \log_{r_t}(\hat{r}_1) = c(1-t)\ v(r_t,t) = \lambda(t)\ v(r_t,t).\end{aligned}$$

E MOTIF GUIDANCE DETAILS

For the sake of completeness, we derive in this section the guidance term in Eq. (2) for the flow matching setting. In particular, we want to derive the conditional vector field $v(x_t, t|y)$ in terms of the unconditional vector field $v(x_t, t)$ and the correction term $\nabla \log p_t(y|x_t)$. Beware, in the following we adopt the time notation from diffusion models, i.e. $t = 0$ for denoised data to $t = 1$ for fully noised data. We therefore need to swap $t \to 1-t$ in the end results to revert to the flow matching notations.

Let's consider the process associated with the following noising stochastic differential equation (SDE)

$$\mathrm{d}x_t = f(x_t,t)\mathrm{d}t + g(t)\mathrm{dB}_t \tag{12}$$

which admits the following time-reversal denoising process

$$\mathrm{d}x_t = \left[f(x_t,t) - g(t)^2 \nabla \log p_t(x_t)\right] \mathrm{d}t + g(t)\mathrm{dB}_t. \tag{13}$$

Thanks to the Fokker-Planck equation, we know that the the following ordinary differential equation admits the same marginal as the SDE Eq. (13):

$$\begin{aligned}\mathrm{d}x_t &= \left[f(x_t,t) - \frac{1}{2} g(t)^2 \nabla \log p_t(x_t)\right] \mathrm{d}t \\ &= v(x_t,t)\mathrm{d}t.\end{aligned}$$

with $v(x_t, t)$ being the probability flow vector field.

Now, conditioning on some observation y, we have

$$\begin{aligned}\mathrm{d}x_t &= v(x_t,t|y)\mathrm{d}t \\ &= \left[f(x_t,t) - \frac{1}{2} g(t)^2 \nabla \log p_t(x_t|y)\right] \mathrm{d}t \\ &= \left[f(x_t,t) - \frac{1}{2} g(t)^2 \left(\nabla \log p_t(x_t) + \nabla \log p_t(y|x_t)\right)\right] \mathrm{d}t \\ &= \left[v(x_t,t) - \frac{1}{2} g(t)^2 \nabla \log p_t(y|x_t)\right] \mathrm{d}t.\end{aligned} \tag{14}$$

As we see from Eq. (14), we only need to know $g(t)$ to adapt reconstruction guidance–which estimates $\nabla \log p_t(y|x_t)$–to the flow matching setting where we want to correct the vector field. Given a particular choice of interpolation x_t from flow matching, let's derive the associated $g(t)$.

Euclidean setting Assume x_0 is data and x_1 is noise, with $x_1 \sim \mathcal{N}(0, \mathrm{I})$. In Euclidean flow matching, we assume a linear interpolation $x_t = (1-t)x_0 + tx_1$. Conditioning on x_0, we have the following conditional marginal density $p_{t|0} = \mathcal{N}\left((1-t)x_0, t^2\mathrm{I}\right)$. Meanwhile, let's derive the marginal density $\tilde{p}_{t|0}$ induced by Eq. (12). Assuming a linear drift $f(x_t,t) = \mu(t)x_t$, we know that $\tilde{p}_{t|0}$ is Gaussian. Let's derive its mean $m_t = \mathbb{E}[x_t]$ and covariance $\Sigma_t = \mathrm{Cov}[x_t]$. We have that (Särkkä & Solin, 2019)

$$\frac{\mathrm{d}}{\mathrm{d}t} m_t = \mathbb{E}\left[f(x_t,t)\right] = \mu(t) m_t.$$

thus

$$\mathbb{E}[x_t] = \exp\left(\int_0^t \mu(s)\mathrm{d}s\right) x_0.$$

Additionally,

$$\begin{aligned}\frac{\mathrm{d}}{\mathrm{d}t}\Sigma_t &= \mathbb{E}\left[f(x_t,t)(m_t - x_t)^\top\right] + \mathbb{E}\left[f(x_t,t)^\top(m_t - x_t)\right] + g(t)^2\mathrm{I} \\ &= 2\mu(t)\Sigma_t + g(t)^2\mathrm{I},\end{aligned}$$

Matching $\tilde{p}_{t|0}$ and $p_{t|0}$, we get

$$\begin{aligned}&\exp\left(\int_0^t \mu(s)\mathrm{d}s\right) x_0 = (1-t)x_0 \\ \Leftrightarrow &\int_0^t \mu(s)\mathrm{d}s = \ln(1-t) \\ \Leftrightarrow &\mu(t) = -\frac{1}{1-t}\end{aligned}$$

and

$$\begin{aligned}&2\mu(t)t^2 + g(t)^2 = 2t \\ \Leftrightarrow &-2\frac{1}{1-t}t^2 + g(t)^2 = 2t \\ \Leftrightarrow &g(t)^2 = 2t + 2\frac{1}{1-t}t^2 \\ \Leftrightarrow &g(t)^2 = \frac{2t}{1-t}.\end{aligned}$$

The equivalent SDE that gives the same marginals is Therefore, the following SDE gives the same conditional marginal as flow matching:

$$\mathrm{d}x_t = \frac{-1}{1-t}x_t\mathrm{d}t + \sqrt{\frac{2t}{1-t}}\mathrm{dB}_t.$$

$\mathrm{SO}(3)$ **setting** The conditional marginal density $\tilde{p}_{t|0}$ induced by Eq. (12) with zero drift $f(r_t,t) = 0$ is given by the $\mathrm{IGSO}(3)$ distribution (Yim et al., 2023b): $\tilde{p}_{t|0} = \mathrm{IGSO}(3)\left(r_t, r_0, t\right)$. We are not aware of a closed form formula for the variance of such a distribution.

On the flow matching side, we assume r_0 is data and r_1 is noise, with $r_1 \sim \mathcal{U}(\mathrm{SO}(3))$, and a geodesic interpolation $r_t = \exp_{r_0}(t\log_{r_0}(r_1))$. We posit that the induced conditional marginal $p_{t|0}$ is *not* an $\mathrm{IGSO}(3)$ distribution. As such, it appears non-trivial to derive the required equivalent diffusion coefficient $g(t)$ for $\mathrm{SO}(3)$. We therefore use as a heuristic the same $g(t)$ as for $\mathbb{R}^d$.

F DESIGNABILITY

We provide details of the designability metric for motif-scaffolding and unconditional backbone generation previously used in prior works (Watson et al., 2023; Wu et al., 2023). The quality of a backbone structure is nuanced and difficult to find a single metric for. One approach that has been proven reliable in protein design is using a highly accurate protein structure prediction network to recapitulate the structure after the sequence is inferred. Prior works (Bennett et al., 2023; Wang et al., 2021) found the best method for filtering backbones to use in wet-lab experiments was the combination of ProteinMPNN (Dauparas et al., 2022) to generate the sequences and AlphaFold2 (AF2) (Jumper et al., 2021) to recapitulate the structure. We choose to use the same procedure in determining the computational success of our backbone samples which we describe next. As always, we caveat these results are computational and may not transfer to wet-lab validation. While ESMFold (Jumper et al., 2021) may be used in place of AF2, we choose to follow the setting of RFdiffusion as close as possible.

We refer to *sampled backbones* as backbones generated from our generative model. Following RFdiffusion, we use ProteinMPNN at temperature 0.1 to generate 8 sequences for each backbone in motif-scaffolding and unconditional backbone generation. In motif-scaffolding, the motif amino

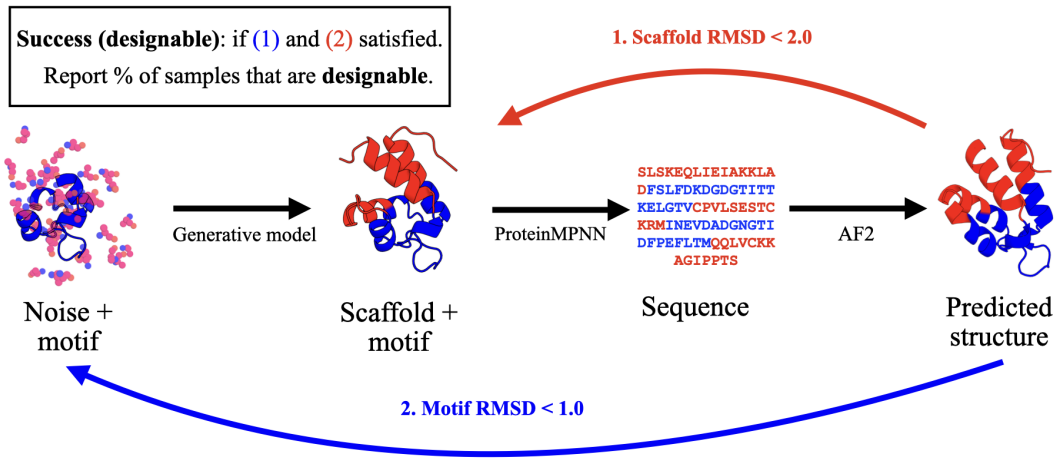


Figure 5: Schematic of computing motif-scaffolding designability. "Generative model" is a stand-in for the method used to generate scaffolds conditioned on the motif. From there, we use ProteinMPNN (Dauparas et al., 2022) to design the sequence then use AlphaFold2 (AF2) (Jumper et al., 2021) in predicting the structure of the sequence. The RMSD is calculated on the scaffold (blue) and motif (red) separately with alignments. A generated scaffold passes designability if the scaffold RMSD < 2.0 and motif RMSD < 1.0.

acids are kept fixed – ProteinMPNN only generates amino acids for the scaffold. The *predicted backbone* of each sequence is obtained with the fourth model in the five model ensemble used in AF2 with 0 recycling, no relaxation, and no multiple sequence alignment (MSA) – as in the MSA is only populated with the query sequence. Fig. 5 provides a schematic of how we compute designability for motif-scaffolding. A sampled backbone is successful or referred to as designable based on the following criterion depending on the task:

- **Unconditional backbone generation**: successful if the Root Mean Squared Deviation (RMSD) of all the backbone atoms is < 2.0Åafter global alignment of the Carbon alpha positions.
- **Motif-scaffolding**: successful if the RMSD of motif atoms is < 1Åafter alignment on the motif Carbon alpha positions. Additionally, the RMSD of the scaffold atoms must be < 2Åafter alignment on the scaffold Carbon alpha positions.

G SET-UP

Training. We train two FrameFlow models. FrameFlow-amortization is trained with motif amortization as described in Sec. 2.1 with data augmentation using hyperparameters: $\gamma_{\text{min}} = 0.05$ so the motif is never degenerately small and $\gamma_{\text{max}} = 0.5$ to avoid motif being the majority of the backbone. FrameFlow-guidance, to be used in motif guidance, is trained unconditionally on full backbones. Both models are trained using the filtered PDB monomer dataset introduced in FrameDiff. We use the ADAM optimizer (Kingma & Ba, 2014) with learning rate 0.0001. We train each model for 6 days on 2 A6000 NVIDIA GPUs with dynamic batch sizes depending on the length of the proteins in each batch — a technique from FrameDiff.

Sampling. We use the Euler-Maruyama integrator with 500 timesteps for all sampling. We sample 100 scaffolds for each of the 24 monomer motifs following the guidelines in the motif-scaffolding benchmark proposed in RFdiffusion. The benchmark has 25 motifs, but the motif 6VW1 involves multiple chains that FrameFlow cannot handle.

Metrics. Previously, motif-scaffolding was only evaluated through samples passing *designability* (**Des.**). For a description of designability see App. F. In addition to designability, we also calculate the *diversity* (**Div.**) of scaffolds as the number of clusters out of the designable samples. Designing

a diverse range of scaffolds increases chances of success in wet-lab validation. This provides an additional data point to check for mode collapse where the model is sampling the same scaffold repeatedly. Clusters are computed using MaxCluster (Herbert & Sternberg, 2008) with TM-score threshold set to 0.5. *Novelty* (**Nov.**) is the average over the TM-score of each sample to its closest protein in the PDB computed using FoldSeek (van Kempen et al., 2022). We only report novelty for unconditional generation since novelty is not necessarily a desirable property for scaffolds and is expensive to calculate for many proteins.

H UNCONDITIONAL BACKBONE RESULTS

We present backbone generation results of the unconditional FrameFlow model used in FrameFlow-guidance. We do not perform an in-depth analysis since this task is not the focus of our work. Characterizing the backbone generation performance ensures we are using a reliable unconditional model for motif-scaffolding. We evaluate the unconditionally trained FrameFlow model by sampling 100 samples from lengths 70, 100, 200, and 300 as done in RFdiffusion. The results are shown in Table. 2. We find that FrameFlow achieves slightly worse designability while achieving improved diversity and novelty. We conclude that FrameFlow is able to achieve strong unconditional backbone generation results that are on par with a current state-of-the-art unconditional diffusion model RFdiffusion.

Table 2: Unconditional generation metrics.

Method	**Des.**(↑)	**Div.** (↑)	**Nov.** (↓)
FrameFlow	0.86	155	**0.61**
RFdiffusion	**0.89**	**159**	0.65

I FRAMEFLOW MOTIF-SCAFFOLDING ANALYSIS

In this section, we provide additional analysis into the motif-scaffolding results in Sec. 3. Our focus is on analyzing the motif-scaffolding with FrameFlow: motif amortization and guidance. The first analysis is the empirical cumulative distribution functions (ECDF) of the motif and scaffold RMSD shown in Fig. 6. We find that the main advantage of amortization is in having a higher percent of samples passing the motif RMSD threshold compared to the scaffold RMSD. Amortization has better scaffold RMSD but the gap is smaller than motif RMSD. The ECDF curves are roughly the same for both methods.

To visualize the diversity of the scaffolds, Fig. 7 shows several of the clusters for motifs 1QJG, 1YCR, and 5TPN where FrameFlow can generate significantly more clusters than RFdiffusion. Each scaffold demonstrates a wide range of secondary structure elements across multiple lengths. To quantify this in more depth, Fig. 8 plot the helical and strand compositions (computed with DSSP (Kabsch & Sander, 1983)) of designable motif-scaffolds from FrameFlow-amortization compared to RFdiffusion. We see FrameFlow-amotization achieves a better spread of secondary structure components than RFdiffusion. App. I provides additional analysis into the FrameFlow motif-scaffolding results. We conclude FrameFlow-amortization achieves much more structural diversity than RFdiffusion.

Lastly, we visualize samples from FrameFlow-amortization on each motif in the benchmark. As noted in Sec. 3, amortization is able to solve 21 out of 24 motifs in the benchmark. In Fig. 9, we visualize the generated scaffolds that are closest to passing designability for the 3 motifs it is unable to solve. We find the failure to be in the motif RMSD being over 1ÅRMSD. However, for 4JHW, 7MRX_60, and 7MRX_128 the motif RMSDs are 1.2, 1.1, and 1.7 respectively. This shows amortization is very close to solve all motifs in the benchmark. In Fig. 10, we show designable scaffolds for each of the 21 motifs that amortization solves. We highlight the diverse range of motifs that can be solved as well as diverse scaffolds.

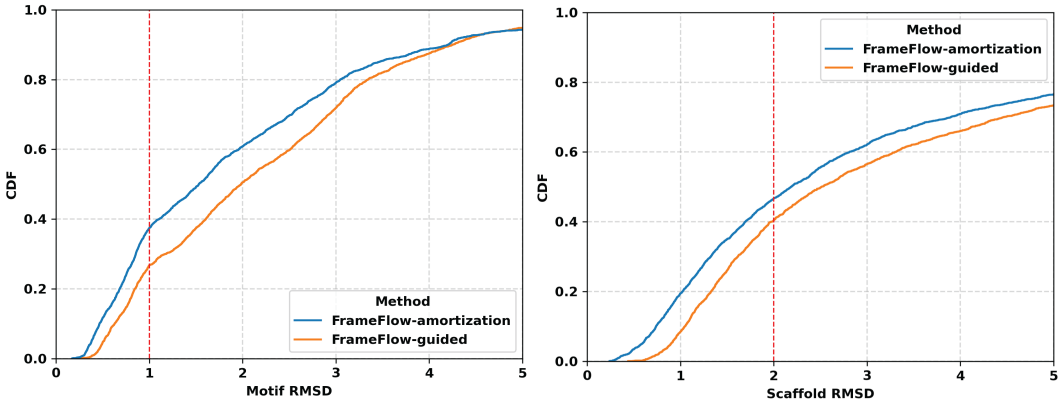


Figure 6: Empirical cumulative density plot of designability RMSD over the motif (left) and scaffold (right) for FrameFlow-amortization (blue line) and FrameFlow-guidance (orange line).

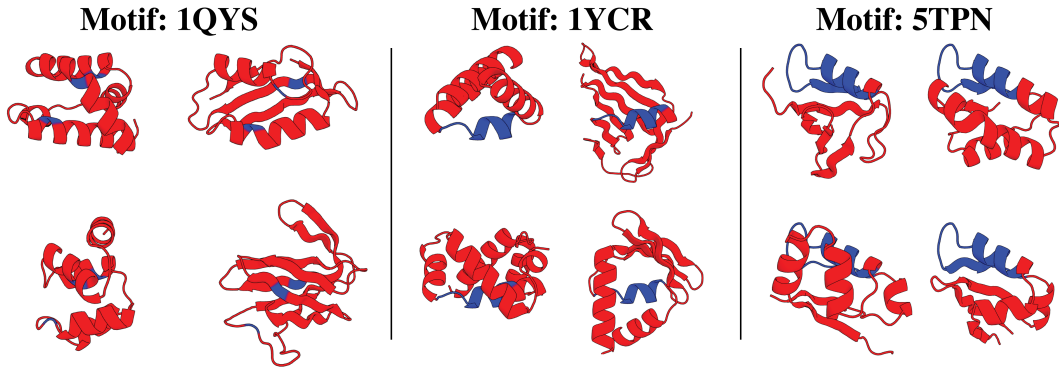


Figure 7: **FrameFlow-amortization diversity.** In blue is the motif while red is the scaffold. For each motif (1QJG, 1YCR, 5TPN), we show FrameFlow-amortization can generate scaffolds of different lengths and various secondary structure elements for the same motif. Each scaffold is in a unique cluster to showcase the samples'structural diversity.

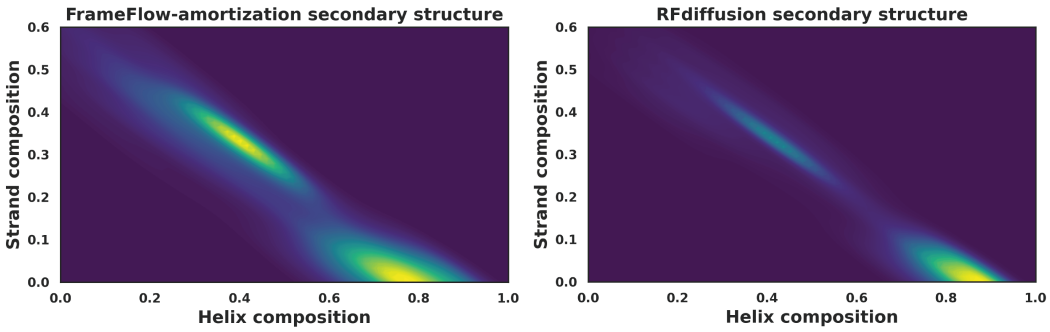


Figure 8: **Secondary structure analysis.** 2D kernel density plots of secondary structure composition of *designable* motif-scaffolds from FrameFlow-amortization and RFdiffusion. Here we see RFdiffusion tends to mostly generate helical scaffolds while FrameFlow-amortization gets much more mixed Strand/Helix scaffolds.

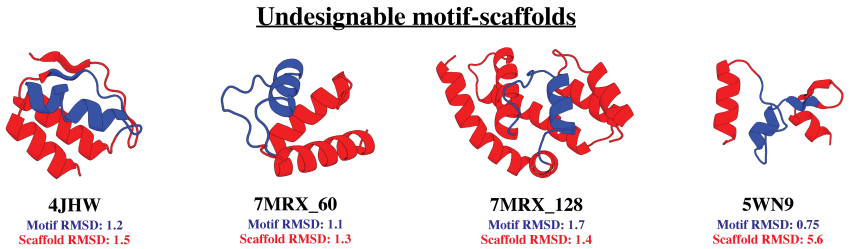


Figure 9: Closest motif-scaffolds from FrameFlow-amortization on the three motifs it fails to solve. We find the > 1.0Åmotif RMSD is the reason for the method failing to pass designability.

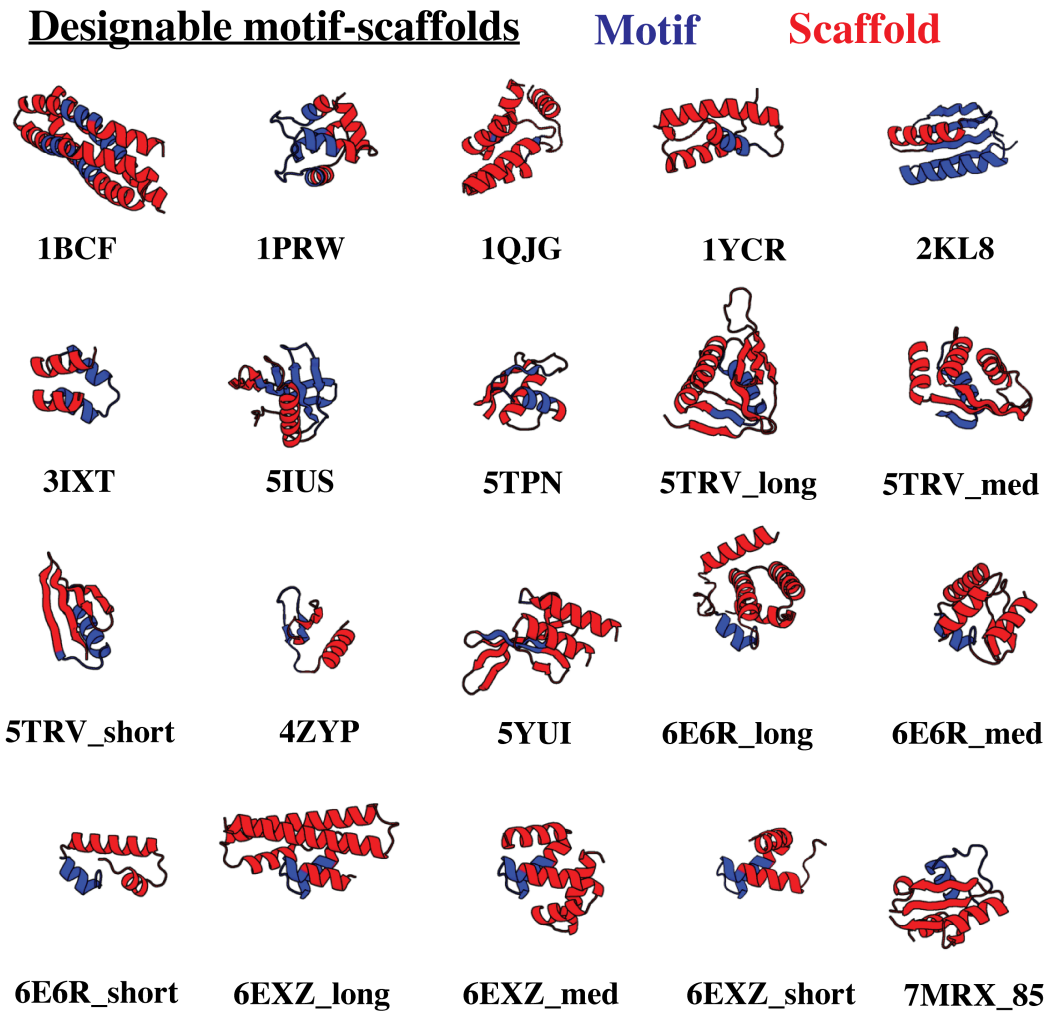


Figure 10: Designable motif-scaffolds from FrameFlow-amortization on 20 out of 24 motifs in the motif-scaffolding benchmark.