1 Multi Polymer Diffusion

Here resides the cookbook for various multi-polymer diffusion examples, highlighting the structure controls that you might not find in other RFdiffusion branches.

Intro

Different types of design tasks that you might want to use this for

- Unconditional generation of multiple biopolymers together
- Motif scaffolding tasks with multiple polymers
- Control of base-pairing secondary structure to design RNA pseudoknots or (mini) DNA origami

You'll generally get the best performance across all tasks by defaulting to the `multi_polymer` config.

Some Example Design Tasks

Example 1: Unconditional RNA generation

```
/path/to/SE3nv.sif /path/to/rf_diffusion/run_inference.py \
--config-name=multi_polymer \
diffuser.T=50 \
inference.num_designs=5 \
contigmap.contigs=['90'] \
contigmap.polymer_chains=['rna'] \
inference.output_prefix='./demo_outputs/RNA_uncond_standard_settings'
```

Example 2: Unconditional design of one protein chain and two DNA chains

```
/path/to/SE3nv.sif /path/to/rf_diffusion/run_inference.py \
--config-name=multi_polymer \
diffuser.T=50 \
inference.num_designs=5 \
contigmap.contigs=['20\ 20\ 75'] \
contigmap.polymer_chains=['dna','dna','protein'] \
inference.output_prefix='./demo_outputs/DNA_prot_uncond_standard_settings'
```

Example 3: RNA riboswitch design with conditioning from Eterna puzzle structure string

Puzzle source: JR_Openknot4_Week6_4RZD

```
((((...............))))(((((((((........)))))))))).......(((((((........)))))))(((...........))).....
```

Because Hydra cannot use parentheses in command-line arguments, replace "(" and ")" with "5" and "3".

```
/path/to/SE3nv.sif /path/to/rf_diffusion/run_inference.py \
--config-name=multi_polymer \
diffuser.T=50 \
inference.num_designs=5 \
contigmap.contigs=['100'] \
contigmap.polymer_chains=['rna'] \
scaffoldguided.target_ss_string='5555................3333555555555........333333333......5555555........333333355
inference.output_prefix='./demo_outputs/RNA_eterna_cond_standard_settings'
```

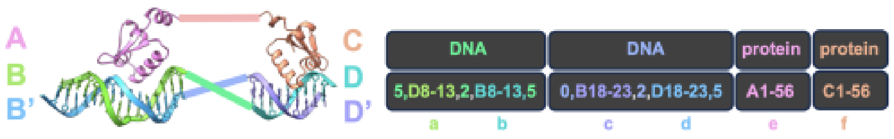
Try this out on more Eterna puzzles! To get the ss-string for a given puzzle, start playing, then right-click → “Copy Structure”.

BUT WAIT! What if you want to insert multiple pseudoknots into your design or insert them into specific positions of certain chains? You can provide a list of secondary structure strings as follows:

```
scaffoldguided.target_ss_string_list=['B1-90:.5555555555555555555..ffffff.33333555....333555....3333333333333333333
'A116-205:.5555555555555555555..ffffff.33333555....333555....333333333333333333555555..tttttt.333333.']
```

where the ranges specify chains and indices of pseudoknot insertion locations in the output contigs.

Example 4: DNA–protein scaffolding, inpaint two DNA chains



The argument `inference.ij_visible` controls which motifs listed in the contigs have their relative orientations locked during inference (credit to David J. for this intuitive system). In the following contact map, lowercase letters denote motifs from the input PDB in the order they occur in the contigs (not to be confused with uppercase chain IDs):

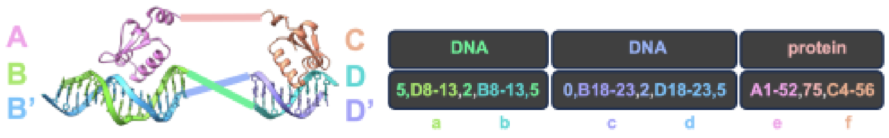
```
10,D8-13,6,B8-13,10\ 10,B18-23,6,D18-23,10\ A1-56,0\ C1-56,0
       a.       b.          c.         d.         e.         f.
```

Motifs `A1-56`, `B8-13`, and `B18-23` (b,c,e) form the first DNA+binder group “bce”, while `C1-5`, `D8-13`, and `D18-23` (a,d,f) form “adf”. The relative orientations of “bce” and “adf” can now vary during inference.

```
/path/to/SE3nv.sif /path/to/rf_diffusion/run_inference.py \
--config-name=multi_polymer \
diffuser.T=50 \
inference.num_designs=5 \
contigmap.contigs=['10,D8-13,6,B8-13,10\ 10,B18-23,6,D18-23,10\ A1-56,0\ C1-56,0'] \
inference.ij_visible='bce-adf' \
contigmap.polymer_chains=['dna','dna','protein','protein'] \
inference.input_pdb='/projects/ml/afavor/test_data/combo_DBP009_DBP010_DBP011_with_DNA_v2.pdb' \
inference.output_prefix='./demo_outputs/DNA_binders_scaffolding_test1_standard_settings'
```

Example 5: DNA–protein scaffolding, inpaint two DNA chains and one protein chain

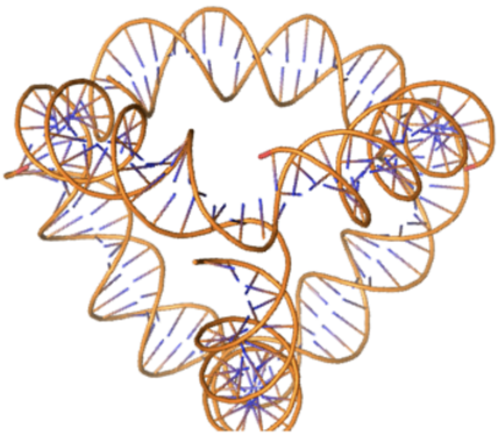
Here, `contigmap.polymer_chains` lists only three chains because binding proteins are merged into one.



```
/path/to/SE3nv.sif /path/to/rf_diffusion/run_inference.py \
--config-name=multi_polymer \
diffuser.T=50 \
inference.num_designs=5 \
contigmap.contigs=['5,D8-13,2,B8-13,5\ 5,B18-23,2,D18-23,5\ A1-52,90,C4-56,0'] \
inference.ij_visible='bce-adf' \
contigmap.polymer_chains=['dna','dna','protein'] \
scaffoldguided.target_ss_pairs=['A1-24,B1-24'] \
inference.input_pdb='/projects/ml/afavor/test_data/combo_DBP009_DBP010_DBP011_with_DNA_v2.pdb' \
inference.output_prefix='./demo_outputs/DNA_binders_scaffolding_test2_standard_settings'
```

The new argument `scaffoldguided.target_ss_pairs` enforces base-pairing of polymer ranges (here `A1-24 B1-24`). Each corresponds to the two full DNA contigs (A,B) in the outputs, each of length 24.

Example 6: DNA origami with symmetric denoising

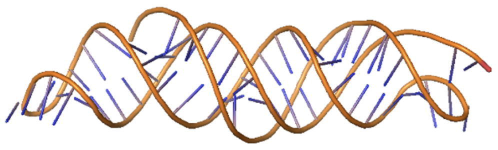


The `scaffoldguided.target_ss_pairs` argument specifies paired ranges in the design; both ranges in each pair must have equal length. The first is 5→3, the second 3→5.

```
/path/to/SE3nv.sif /path/to/rf_diffusion/run_inference.py \
--config-name=multi_polymer \
diffuser.T=50 \
inference.num_designs=5 \
contigmap.contigs=['60\ 60\ 60\ 60'] \
contigmap.polymer_chains=['dna','dna','dna','dna'] \
scaffoldguided.target_ss_pairs=['A1-20,B1-20','A21-40,C21-40','A41-60,D41-60','B21-40,D21-40','B41-60,C41-60','C1-
inference.symmetry='d2' \
inference.output_prefix='./demo_outputs/DNA_origami_standard_settings'
```

By default, strands are antiparallel; you can, however, define parallel orientations for creative topologies—explored next.

Example 7: RNA design with triple helix

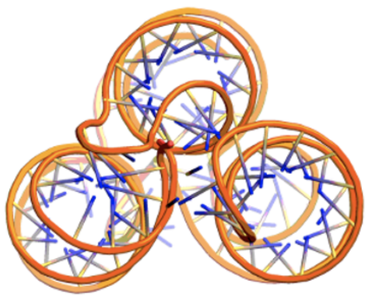


Just as in the previous example, we can use base-paired ranges of sequence to control RNA topology. By default, paired regions are antiparallel, but specific orientations can be assigned (e.g., triple helices with parallel/antiparallel combinations).

```
/path/to/SE3nv.sif /path/to/rf_diffusion/run_inference.py \
--config-name=multi_polymer \
diffuser.T=50 \
inference.num_designs=5 \
contigmap.contigs=['75'] \
contigmap.polymer_chains=['rna'] \
scaffoldguided.target_ss_pairs=['A5-20,A55-70','A55-70,A30-45'] \
scaffoldguided.target_ss_pair_ori=['P','A'] \
inference.output_prefix='./demo_outputs/Triple_helix_test'
```

Each orientation in the list `scaffoldguided.target_ss_pair_ori` corresponds to the element at the same index in `scaffoldguided.target_ss_pairs`.

Example 8: Control of RNA tertiary structure with multi-contact specification



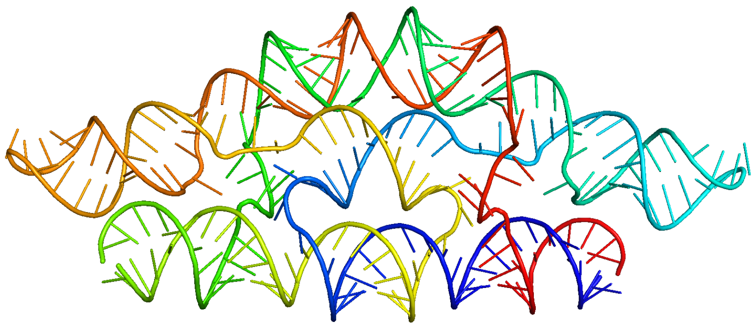
We can "staple" distal loops in RNA pseudoknots together by specifying regions of multi-base contacts using `scaffoldguided.force_multi_contacts`. Secondary-structure strings cannot encode beyond simple two-base pair configurations, so this feature enables higher-order tertiary interactions. We can also force loop placement via `scaffoldguided.force_loops_list`.

```
/path/to/SE3nv.sif /path/to/rf_diffusion/run_inference.py \
--config-name=multi_polymer \
diffuser.T=50 \
inference.num_designs=5 \
contigmap.contigs=['80'] \
contigmap.polymer_chains=['rna'] \
scaffoldguided.target_ss_pairs=['A5-15,A25-35','A45-55,A65-75'] \
scaffoldguided.force_multi_contacts=['A19,A61,A20','A59,A21,A60'] \
scaffoldguided.force_loops_list=['A38-42'] \
inference.output_prefix='./demo_outputs/loop_touch_test'
```

Example 9: Pseudocyclic symmetry using procedurally generated base-pair patterning

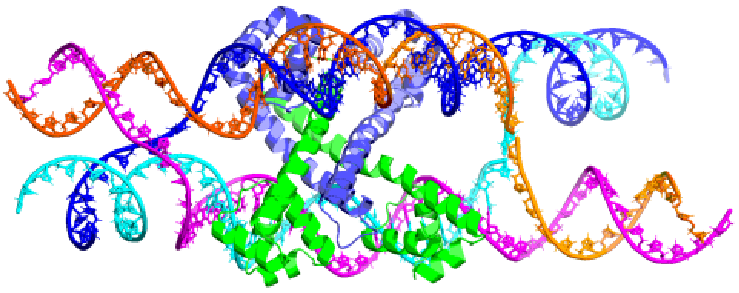
Below are arguments used to create pseudo-symmetry in a single chain forming a cyclic-symmetric shape (e.g., C_2 pseudocycle).

```
/path/to/SE3nv.sif /path/to/rf_diffusion/run_inference.py --config-name=multi_polymer \
inference.ckpt_path='/models/all_na_ss_cond/train_session2024-07-08_1720455712_BFF_5.00.pt' \
diffuser.T=50 \
inference.num_designs=5 \
contigmap.contigs=['240'] \
inference.pseudo_symmetry='c2' \
inference.n_repeats=2 \
```



```
scaffoldguided.target_ss_pairs=['A4-5,A237-238','A7,A236','A11-12,A230-231','A18-27,A137-146','A44-47,A72-75','A4
'A63-69,A49-55','A72-75,A44-47','A90-99,A197-206','A110-112,A130-132','A115-115,A127-128','A117-118,A124-125','A1
'A127-128,A115-116','A130-132,A110-112','A137-140,A24-27','A141-146,A18-23','A164-168,A191-195','A169-175,A183-18
'A191-195,A164-168','A197-206,A90-99','A210-214,A82-86','A215-219,A77-81','A230-231,A11-12','A236,A7','A237-238,A
contigmap.polymer_chains=['rna'] \
inference.output_prefix='./outputs_2025-02-03/pC2_test01__BFF_5.00'
```

Example 10: De novo Holliday junctions using strand exchange



We can use symmetry and strand exchange to design Holliday-junction-style complexes. Chain and index specifications in `scaffoldguided.target_ss_pairs` refer to chain IDs and indices in the output structure defined by contig topology.

```
/path/to/SE3nv.sif /path/to/rf_diffusion/run_inference.py \
--config-name=multi_polymer \
diffuser.T=50 \
inference.symmetry='c2' \
inference.num_designs=3 \
contigmap.inpaint_seq=['D60','D57','D58','D11','D15','D19','D8','D61','D18','A9','A59','A62','A8','A4','A5'] \
inference.num_designs=5 \
inference.ckpt_path='/models/all_na_ss_cond/train_session2024-07-08_1720455712_BFF_5.00.pt' \
contigmap.contigs=['A1-61,60,D14-65\ 15,B6-12,4,F1-8,15\ 15,E7-14,4,C4-10,15\ A1-61,60,D14-65\ 15,B6-12,4,F1-8,15
inference.ij_visible='acf-bde-gil-hjk' \
contigmap.polymer_chains=['protein','dna','dna','protein','dna','dna'] \
scaffoldguided.target_ss_pairs=['B1-10,F40-49','B40-49,F1-10','B16-34,C16-34','E16-34,F16-34','C1-10,E40-49','C40
inference.input_pdb='/projects/ml/afavor/test_data/DBP35opt_DBP48.pdb' \
inference.output_prefix='./outputs_2025-02-03/DBP_scaffolding_test06__BFF_4.00'
```

Example 11: Sequence specification and sequence design

These are two new features that I've added recently, so I'm placing two examples at the top of the wiki page so that they're the first thing people see. Anyway, we can now specify the sequence of our structures to be whatever we want!

Additionally, I've trained the model to do sequence prediction, so we can decode a sequence during the denoising trajectory (this allows us to generate outputs with all of the base atoms rendered for NA stuff, as well as nice sidechain interactions for protein stuff).

The default behavior throughout RFdiffusion is to keep the sequence of diffused regions masked during the trajectory, even if the outputs seem to have residue labels. RoseTTAfold must "see" sequence labels in order to generate sidechains, so I added a flag, `inference.update_seq_t=True`, which allows the model to see either a user-specified sequence or the model's predicted sequence from the previous timestep. This gives us sidechains, and it is super cool.

There are two ways to control the sequence info during a trajectory:

- Turn on full-sequence visibility at some timestep towards the end of the trajectory, using `inference.show_seq_under_t=15`.
- Gradually decode a random selection of positions at each step below some point in the trajectory, using `diffuser.aa_decode_steps=40`.

Both methods work nicely, so test them both during your design process, and let Andrew know if you find that one works better! Examples using both methods are shown below.

Unconditional design of RNA and protein While specifying the RNA sequence, letting the model design the protein sequence, and gradually revealing various sequence positions over the course of the last 40 steps:

```
/path/to/SE3nv.sif /path/to/rf_diffusion/run_inference.py --config-name=multi_polymer \
diffuser.T=50 \
inference.num_designs=3 \
contigmap.contigs=['43\ 20\ 75'] \
contigmap.polymer_chains=['rna','rna','protein'] \
inference.set_sequence=['A1-43:GGAUGUACUACCAGCUGAUGAGUCCCAAAUAGGACGAAACGCC','B1-20:GGCGUCCUGGUAUCCAAUCC'] \
inference.update_seq_t=True \
diffuser.aa_decode_steps=40 \
inference.output_prefix='./demo_outputs/RNA-prot_seq-spec_and_seq-design_standard_settings'
```

Unconditional design of DNA and protein While specifying the DNA sequence, letting the model design the protein sequence, and letting the model see for the last 15 steps (currently throws an error but works with autoregressive decoding):

```
/path/to/SE3nv.sif /path/to/rf_diffusion/run_inference.py --config-name=multi_polymer \
diffuser.T=50 \
inference.num_designs=3 \
contigmap.contigs=['33\ 33\ 75'] \
contigmap.polymer_chains=['dna','dna','protein'] \
scaffoldguided.target_ss_pairs=['A11-23,B11-23'] \
inference.set_sequence=['A11-23:TAGCAGGATGTGT'] \
inference.assume_canonical_pair_seq=True \
inference.update_seq_t=True \
inference.show_seq_under_t=15 \
inference.output_prefix='./demo_outputs/DNA-prot_seq-spec_and_seq-design_standard_settings'
```

Notice in the DNA example above, only one of the dsDNA chains is specified. Since we specified the paired regions using `scaffoldguided.target_ss_pairs=['A11-23,B11-23']`, the model knows which bases should be paired. Then, we can use the flag `inference.assume_canonical_pair_seq=True` to fill in canonical base-pair partners automatically.

2 Further Reading

Secondary Structure Strings and Hydra

We can use several symbol pairs to denote which of two bases being paired is 5'-led or 3'-led. Unfortunately, due to Hydra's constraints on command-line tokens, we cannot use standard DBN conventions and must exclude the following symbols: `(`, `)`, `[`, `]`, `{`, `}`, `<`, `>`. However, the following symbols can be used instead to control hierarchical pseudoknot specification:

5'-led	3'-led	Explanation
5	3	(5'$\rightarrow$3' and 3'$\rightarrow$5')
i	j	(index convention)
f	t	("from" and "to")
b	e	("begin" and "end")
A	a	("begin" and "end")

The code accepts any of these symbols to denote base pairs using the dot-bracket-notation paradigm. However, Hydra cannot parse parentheses, braces, or brackets in command-line arguments. If your topology requires multiple distinct open/close pairs (as with pseudoknots), simply replace symbols as long as they remain distinct.

Polymer Contigs

If you've gotten this far, you've probably already seen the arguments such as:

```
contigmap.polymer_chains=['dna','dna','protein']
```

To control polymer classes generated, specify the polymer type for each chain in the contig line; otherwise, the model makes assumptions that may yield odd results (e.g., threading nucleic backbones through protein motifs). Future updates may automate this behavior, but for now specify polymer chains manually.

A Note on the Different Configs

Different configs and their intended use cases:

- **multi_polymer:** main config; handles motif scaffolding and secondary-structure conditioning.
- **RNA_ss_cond:** similar to above, but loads a checkpoint fine-tuned on more RNA samples.
- **RNA_uncond:** older configuration predating SS-conditioning. Produces diverse unconditional designs but with less designable backbones.

In practice, *multi_polymer* works best across all polymer classes and tasks — truly a "one-model-to-rule-them-all" scenario.

A Note on Checkpoints

Many checkpoints are available, but the `multi_polymer` config generally suffices. Alternative checkpoints can be found at:

```
/models/
```

The file `MODEL_DESCRIPTIONS.md` describes them in detail.

Arguments that specify residues Chain–resi pairs (e.g., "A23") specify that something should happen to a given residue. Sometimes we use this to refer to the chain–resi pairing in the input PDB (for example, in the `contigmap.contigs` argument). Other times we refer to a location within the newly generated structure (for example, in `scaffoldguided.target_ss_pairs`).

Residue Specification Arguments

Various diffusion arguments use residue specifications to reference positions in input or output structures.

Reference structure	Argument
Input PDB	`contigmap.contigs=['B1-14,5,...,H1-9,0']`
Output design	`scaffoldguided.target_ss_pairs`, `scaffoldguided.target_ss_string_list`, `contigma`

3 Bonus Problems

Scaffolding a G-quadruplex

(Placeholder for future examples — to be implemented.)

A Note on PyMOL's Cartoon Representation

(IMPORTANT) As it currently stands, RFdiffusion outputs lack sidechains in non-motif regions. This is particularly problematic for nucleic acids: PyMOL refuses to render nucleic acid cartoons if sidechains are missing. Therefore, Andrew recommends inspecting outputs in ChimeraX or using an alternative PyMOL scheme (he has a script for this — ask him if interested).

TL;DR: If outputs seem invisible in PyMOL, it's not a bug or a feature — just a rendering limitation.

To-Do

If anything here is unclear, please let Andrew know! The only way to discover what works best is for users to create new design challenges — play around and share.

End of document.