**PAS-mes protocol**

Since the active site engineering worked well, start with this method first.

1. Diffuse residues within 8 angstroms of substrate:

[36, 37, 38, 39, 40, 41, 42, 157, 158, 159, 160, 167, 169, 174, 177, 178, 179, 180, 181, 182, 185, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 203, 205, 206, 207, 209, 210, 213, 224, 225, 226, 227, 266, 268, 269, 270, 279, 280, 281, 282, 283, 317, 318]

Command:

*python run\_inference.py inference.deterministic=False diffuser.T=200 inference.output\_prefix=output/ligand\_protein\_motif/PAS\_mes\_ inference.input\_pdb=input/PAS\_mes\_complex.pdb contigmap.contigs=[\'1-10,A5-35,5-10,A43-156,60-80,A228-265,15-25,A284-316,15-30\'] contigmap.length="150-350" inference.ligand=MES inference.num\_designs=5 inference.design\_startnum=0*

\*\*Since large chunks from 157 to 227 is within 8A of target, diffuse entire part of protein.\*\*

2. Protein MPNN then alphafold3 for prediction

a) Select 2 best structures from 8A for protein MPNN then alphafold3 prediction for all 4 sequences.

- 8A: diffused structures 1 and 3 chosen

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8A results:

structure 1 – protein mpnn sequence id3 and id4 chosen.

Results of alignment (3/6/2024):

id3: RMSD 1.077, huge, very open pocket, might bind but unlikely to be able to catalyse, **moved to next step**.

id4: RMSD 1.575, huge, very open pocket, might bind but unlikely to be able to catalyse, steric clash. REJECTED.

Structure 3 – protein mpnn sequence id 5 and 8 chosen.

Results of alignment (3/6/2024):

id5: RMSD 5.434, smaller pocket, steric clash, manually adjusted substrate and **moved to next step** .

id8: RMSD 5.581, smaller pocket, steric clash, REJECTED.

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b) Also do protein MPNN only only PAS\_mes\_complex template, 2 sequences for alphafold prediction.

PAS\_mes\_complex: protein mpnn id3 and id7 sequences selected.

Results of alignment (3/6/2024):

id3: 0.574 RMSD, tight pocket. REJECTED

id7: 0.602 RMSD, tight pocket, almost identical to id3, no point moving both, **moved to next step**.

3. Ligand MPNN of the best aligned structures using 3 different ligand MPNN parameters for evvery structure:

* 1. Normal
  2. Higher temperature: 0.25
  3. Side-chain packing

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Ligand mpnn results, 1 sequence chosen for each parameter using overall confidence, so 9 sequences total, including mpnn only design, for AF3.

See excel for selected sequences, scores and filenames.

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4. Docking results

see Excel.