**Van der Waals packing facilitates membrane protein association**

**Abstract**

- TBD

**Graphical Abstract**

- Have a nice figure of how my design algorithm works, maybe tie it into the figure 1 (extracted geometries), finally output the protein and show fluorescence

- I also think visually for my presentation, having some sort of pymol driven video would be nice

**Premise/Intro**

- packing contribution to folding and association in membrane proteins is not well understood

- Look at other vdW and design papers to see how they structure this; Mravic et al. in particular

- other forces have been studied using dimers as a tractable model system

- protein design as a tool to study dimeric protein-protein interactions in a variety of systems

**Figure 1**

A diagram of a graph

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Figure 1. Membrane protein dimer design. (a) Helices within close contact (define how) were extracted from all membrane protein dimers submitted to the PDB in (date; 2019). Orientations of Proteins in membranes (OPM, cite) was used to only choose the proteins found in membranes. These geometric terms are plotted and overlaid over the density of these in space. (b) Design algorithm…

* Membrane protein design using the membrane protein geometric landscape from the pdb:
  + X proteins were extracted from orientations in membrane proteins
  + Any two helices in close contact (define) were extracted from each membrane protein structure (total number), and the helical geometric information was extracted using MSL
  + These helical geometries were used to determine a density plot of the potential designable space for membrane protein dimer geometries (figure)
  + Protein design using MSL (need to decide how much detail here; most should go into methods)
  + Designed x proteins with a range of expected stabilities
  + Talk about the design regions (GAS, right, left)

**Figure 2**

**A diagram of a dna sequence

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Figure 2. Controlling for expression and insertion variability. (a) [Design algorithm name] was run on a poly-leucine backbone, allowing for 8 variable amino acid positions. GASright and right handed positions are identical, while left handed positions are based on knobs into holes packing motifs found within coiled coils. (b) TOXGREEN fuses the designed protein to maltose binding protein (MBP) in the periplasm and ToxR, a dimeric transcription factor, in the cytoplasm. Dimerization of designs results in the dimerization of ToxR, and subsequently the transcription and translation of GFP as a readout of association strength.

* Describe experimental protocol and how TOXGREEN works with references to TOXCAT and TOXGREEN literature
  + Should we also add in detail for sort-seq here?
  + If sort-seq is added, include a figure that details how we convert the reconstructed fluorescence to percent GpA using TOXGREEN

**Figure 3**

A collage of graphs and diagrams

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Figure 3. A) The normalized fluorescence plotted against the design energy score for all of the designed sequences in the dataset. The plot is separated by the geometric region of design with GASright in green, Left in yellow, and right in purple. B) The dataset is filtered for designs where at least 1 clash and void mutant is also found to fluoresce. The WT (dark green) fluorescence distribution is compared to the fluorescence distributions of their corresponding mutants. C) Separated graphs for normalized fluorescence against the design energy score in A.

* Fluorescence distribution vs design plots for all sequences
  + Haven’t checked significance between designs and mutants yet, but that info would go here
  + Could show regression graphs for all of these sequences
  + Talk about GASright being the most stable, then left, then right
    - Importance of hydrogen bonding for membrane protein stability
  + Design energy score appears to better capture the GASright than the other structures
    - Mention potential for utilizing other energy terms (electrostatics)

**Figure 4**

A graph of a graph with numbers and dots

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Figure 4. Converting fluorescence to free energy.

* Detail here for how we convert from fluorescence to delta G
  + Cite Gladys paper
* Talk about how we trimmed to this dataset:
  + This image is for:
    - At least 1 clashing mutant < 35% OR the difference between the WT and mutant is > 50%
  + Give more information about clashing mutations here (probably also earlier in the design section)
* Talk about the impact of van der Waals packing in left and right versus GASright