Questions for Alessandro about project:

Why is it important to have free energy? I’m trying to find a way to make aim 2 sound compelling (Samson suggested switching the orders of my aims to make it sound more evenly divided in terms of pitching the project)

* Dissect contribution of vdW is difficult (underpacked is penalized, but may not drive); packing does not drive soluble folding, but underpacked proteins are costly (cavities and water inside are costly)
* How much stability is packing provided?

What about Entropy? How do we know that it’s not going to be entropy?

* Entropy changes with temperature, so maybe changing temperature may help
* Calorimetric measurements would measure the heat of reaction/the enthalpic component
  + Entropic cost: protein monomer more free, sidechains at dimer interface are very fixed, lipids lose conformational entropy (all of these need to be overcome)
  + Surface area and volume are good points to talk about
* Delta E between monomer and dimer for Samantha’s data

How should I explain our understanding that we can gain from geometries (if we can only design 20 stable structure for bad geometries and 100 for good geometries, what does that say?)

What other data should I include?

* Maybe don’t put in aim 1 flow; say given backbones, I can produce sequences with sidechain packing, and change the packing quality
  + Think about how to penalize cavities (vdW doesn’t do that)
    - Read about RosettaMembrane to determine a weighted function that will allow me to fit data to the best predicting function
    - Maybe use these for training a function?
* At end of aim, say I haven’t mentioned how to pick the backbones, and want to present some experimental analysis for this because I want to pick favorable backbones
  + Make sure I think about scrambling sequences as a way to determine the importance of sidechain packing
    - What is new is that people have done this analysis, people have focused on the novelty of regular helices (distortions of the helices are costly; small proteins tend to be more straight) and with two more degrees of geometry
      * What are preferred geometries for canonical helices
        + Things that are crossed pack better (more likely to form knobs into holes packing)

Statistical preference vs packing preferences (which gives bias as well from membrane)

To determine the difference between our dataset and the nonpacking dataset, may be possible to determine the statistical dataset (remove any of the distance related ones and extract the others to determine all of the possible vectors for it)

Helices should be following that distribution of angles possible in a sphere, but outside of the packing

* + Maybe show an image of non-regular vs regular helices
    - Ask what is the purpose of doing/showing something and what am I trying to achieve?
    - Prediction part of the membrane protein explanation is not as important; we don’t understand much about the biophysical properties which is what should be emphasized instead, and then the folding aspect of these
  + Also maybe a slide that says that what folds a soluble proteins is the hydrophobic effect, but this is different in membrane proteins
    - Only insertion is driven by hydrophobic effect (then the rest of Alessandro’s slide is important; is difficult to measure how much vdW packing, which I have the way to do; AND I can do this in a general system) (in Alessandro’s current opinion review if need more info)
      * Reason why polyleucine is good: doesn’t dimerize; any changes in dimerization should be due to changes in sidechain packing
        + Would it be a good idea to run a run on just polyleu and see what the difference between monomer and dimer is?
      * Data to support the idea that packing is a driving force is important
        + General understanding of packing as a driving force is important!!!
      * Make a good thermodynamic argument

**What papers should I read to prep for questions?**

Read through Samantha’s Thesis on sort-seq

FRET?

**What are good papers that I have read to refer to when answering some questions?**

Quenching paper for answering how to determine oligomeric state

Support for assumption that point mutants does not destabilize the monomeric state

Other more sensitive tools for studying vdW packing: What tools and methods have been used in the past? (basically, what other vdW research is there?)

Design papers: What points may I need to bring up from these? Standardized methods used in those?

Choosing a subset of geometries/amino acids and reasoning behind that?

Why high-throughput methods give good data? And to what extent are they believable? (not sure if there are papers for this, but might be nice to search for and read some of the papers Samantha sent me; I think it’d be better to read through Samantha’s thesis to answer some questions)

Read (and maybe ask Josh) about how to explain the sensitivity profiles?

Poly-leu and standardization background?

FRET and alternative biophysical methods

Mutagenesis profile: first show one of the old plots for disruption, then say we graph this data as the average of each position for how important each position is to association

* Use experimental data with the mutations (how different mutations change association)
  + Made restraints for docking, want two amino acids to be very close
    - Docking with and without restraints

(check out some stuff from Samantha’s thesis to be sure how to explain this, maybe take some images from it)

Packing is a consequence…something like that to make necessary sound more important

Geometries: how important are they? And how important are the positions at the interface? Should it be standardized or should the numbers of positions be standardized? What

How to control and test for things in FRET?

How can you know how random it is based on fluorescence?

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