* How to detect oligomerization?
  + Weakly stable surface
    - **\*** Two ways I can think of to explain the theoretical basis, of why this is worth trying
      1. It has been observed in other proteins that there is a stabilization upon binding. For example, many intrinsically disordered proteins fold upon binding
         * **\*** though this may be obvious if I understood more, why does folding upon binding imply stabiliziation upon binding?
      2. If the oligomerization reaction is in equilibrium, and the native form is for example a trimer, then we expect the trimer to have lower energy than the three proteins separated. If the monomer is a barrel in the trimer, and we look at the structure of the barrel individually (no change of shape), the high energy should be local to the interface, where the native bonds have been severed. If a protein exists in the membrane as mixed monomer and oligomer, then we won't be able to detect it in this way, since that suggests that the monomeric form is of comparable energy to the oligomeric form (the concentration of the protein within the membrane is a factor too and other stuff that I don't know about), but if it exists in a membrane primarily as an oligomer, if the monomeric form is of two high energy for it to have much of a frequency, then this is worth trying
    - Naveed Jackups Liang (NaJaLi) have tried it
      * Used a knowledge-based potential to predict weakly stable regions, and successfully predict interfacial sites
      * Their method works almost as well from sequence as it does from structure, which makes it usable
        + It does this by considering only the transmembrane strands, which can be predicted fairly well from sequence

**\*** How do they make sure they have the strand registry right, in the paper on predicting oligomeric interfaces? Do they use TMSIP for that? Certainly by the time they publish that structure prediction paper they can do this with TMSIP

* + - * Two functions: predict oligomerization *state* (yes/no), predict oligomerization *interface*
      * A complication, though, for prediction of oligomerization *state:*
        + Explain what in-plugs and outclamps are
        + Since they're only looking at the strands, there are *two* disturbances to native structure in their model: separation of the complex, and removal of inplugs/outclamps
        + When they look at just predicted stability (specifically, predicted melting temperature), the high energy barrels are those in oligomers, as well as those with inplugs and outclamps
        + So, the TMSIP energy function predicts that removing these inplugs is a disturbance to the native state on par with separating the oligomer

**\*** While I feel like I can explain why we'd expect oligomeric interfaces to have high energy, this isn't something I'd necessarily expect. Thermodynamic hypothesis means that the native structure, with the inplug inside the protein, is the lowest energy structure that *contains the inplug*It doesn't necessarily say anything about the stability if it were possible for the inplug to be removed, that is, if the inplug was not covalently linked. As far as I understand, it's possible for a protein to have an inplug inside of it that is destabilizing (compared to if the inplug was cleaved off), as long as the inplug would be *more* destabilizing if it went anywhere else.  
So, I'm prepared to just say that the energy function gives high energy to the proteins with inplugs

* + - * + NaJaLi's solution: look at *variance* of TMSIP energies of the strands of a protein.

**\*** I *think* it's variance. Maybe you should take a look at the formula in the paper with me. It's pretty nonsensical looking, but it does look like someone tried to write the variance formula but made some typos.

This does pretty well at separating the oligomers from the proteins with inplugs and outclamps

Why it separates them from those with inplugs seems easy to explain: the inplug contacts every strand, thoguh perhaps not equally. Whereas, the oligomerization interface is only over part of the protein.

* + - * What proteins does NaJaLi strand variance predict to be oligomers?
        + Maltoporin is a predicted oligomer (as are others of similar structure).

Maltoporin is an oligomer in vivo, and the complex is extraordinarily stable (**\*** I have on file somewhere the temperature you need to get it to dissociate in sodium dodecyl sulfate, I think)

* + - * + OMPLA is not predicted an oligomer

forms oligomer only under certain conditions, so, under other conditions, it is stable as a monmer

* + - * + FepA, but not FhuA, predicted oligomer (**\*** they're <26% seq identical, but they're both iron-transporting proteins and look kind of similar)

Both reported oligomers in the experimental literature, but crystallize as monomers

* + - * + **\*** It really seems like there are interesting interpretations of these facts, regarding what kinds of proteins have weakly stable sites. Like, from the lack of oligomers in the crystal structure, might we have thought, without this study, that FepA and FhuA may not have weakly stable sites? In crosslinking studies of TonB, they've found FepA dimers cross-linked with TonB, so we might have thought that TonB held them together?
      * Inplugs and outclamps also complicate prediction of oligomerization *site*
        + Maltoporin and relatives all have a constriction loop contacting the side of the barrel directly across from the interface
        + However, in almost all cases, the interface signal dominates. In one case, it does not seem to
      * Conclusion - searching for weakly stable strands using TMSIP responds to inplugs, outclamps, and oligomers, and looking for high-energy strands to make a prediction of interfacial site has the same problem. However, despite these complications, it can be done.
    - Ezβ and hydrophobicity
      * The theory
        + Depends upon hypothesis that protein protein interfaces have higher energy of lipid interaction
        + Lipid-facing residues very hydrophobic, interior residues not necessarily hydrophobic: Hayat et. al's exposure propensity scale correlates .7-.8 with hydrophobicity-based AAindices

Buried in interface like buried in protein?

* + - * + **\*** I remember reading something about interfaces often having polar "hot spots"?
      * In practice
        + Hydrophobicity alone does not predict interface (Ezβ paper)

\* I also know that measure of variation in hydrophobicity (moment magnitude) does not predict, but this is unpublished, how much can I talk about it?

* + - * + Regions of elevated Ezβ predict interface, and variability predicts oligomer, only if loops are included in calculation
        + Why not work with no loops?

Contradicts initial intuition

Hayat: interfaces slightly *more* hydrophobic on average. May still be polar hot-spots, but looking for an area elevated on average (of hydrophobicity, and apparently Ezβ) will not work

* + - * + Why work with loops?

Phale: L2 latching loop. Polar and inside the membrane, buried in a neighboring residue

* + - * Conclusion: While weakly stable strands predict oligomerization, this weakly stableness cannot be attributed ONLY to lipid interaction.
        + TMSIP: depth and interstrand interactions
        + Ezβ: only depth
      * Energy from interactions within the protein also has to be taken into account