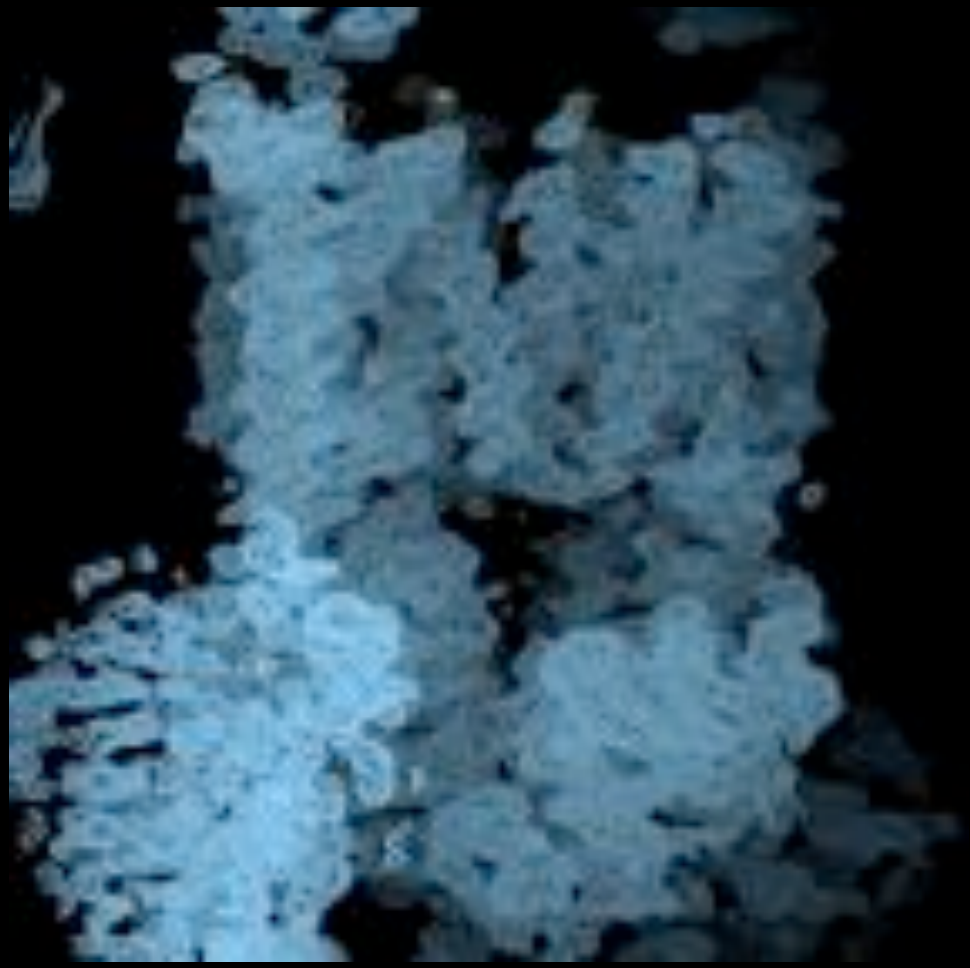
Structural Biology of Membrane Proteins: Are There Any Rules?



D.C. Rees
Caltech/HHMI
NIH Roadmap Meeting

Are there any rules?

- no magic bullets (shots on goal)
- proteins that are polydisperse crystallize less well (M. Wiener)
- lipids are really important (R. Kaback)
- there is no substitute for good (diffraction) data

Are there any problems?

- eukaryotic membrane protein production
- complexes
- membrane protein stability

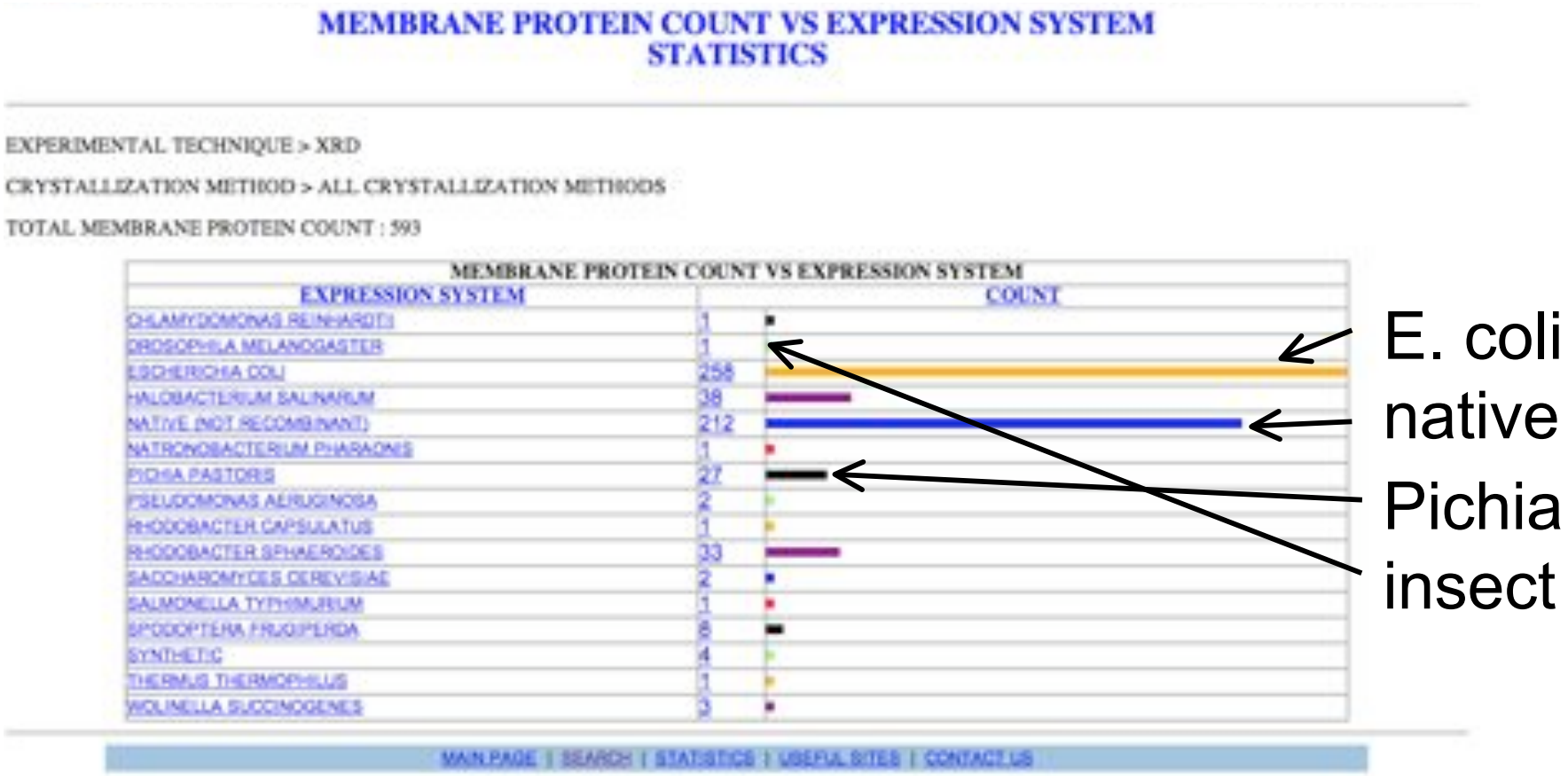
Outline of a (membrane protein) (crystal) structure analysis

- target identification (1 versus many homologues)
  target selection and number of targets
- natural sources or cloning & expression (recombinant)
- solubilization (detergents and other membrane mimetics)
- purification and characterization
- crystallization
- structure determination
- analysis

need to design experimental approach to maintain manageable number of explored variables

will discuss our experiences with a funnel-type approach

Sources of membrane proteins



MEMBRANE PROTEIN COUNT VS EXPRESSION SYSTEM STATISTICS

EXPERIMENTAL TECHNIQUE > XRD

CRYSTALLIZATION METHOD > ALL CRYSTALLIZATION METHODS

TOTAL MEMBRANE PROTEIN COUNT : 593

MEMBRANE PROTEIN COUNT VS EXPRESSION SYSTEM

EXPRESSION SYSTEM	COUNT
CHLAMYDOMONAS REINHARDTII	1
DROSOPHILA MELANOGASTER	1
ESCHERICHIA COLI	258
HALOBACTERIUM SALINARUM	38
NATIVE (NOT RECOMBINANT)	212
NATRONOBACTERIUM PHARAONIS	1
PICHIA PASTORIS	27
PSEUDOMONAS AERUGINOSA	2
RHODOBACTER CAPSULATUS	1
RHODOBACTER SPHAEROIDES	33
SACCHAROMYCES CEREVISIAE	2
SALMONELLA TYPHIMURIUM	1
SPODOPTERA FRUGIPERDA	8
SYNTHETIC	4
THERMUS THERMOPHILUS	1
WOLINELLA SUCCINOGENES	3

MAIN PAGE | SEARCH | STATISTICS | USEFUL SITES | CONTACT US

E. coli

native

Pichia

insect

Guideline: don't break up the team

prokaryotic systems for prokaryotic proteins

eukaryotic systems for eukaryotic proteins

in vitro systems for ?

http://www.mpdb.ul.ie/

Exceptions:

structures of eukaryotic membrane proteins from prokaryotic expression systems?

not from our lab……

human 5-Lipoxygenase activating protein (FLAP)
Ferguson *et al. Science* **317**, 510 (2007)
pET28a in *E. coli* BL21 (DE3)

Expression, Growth, and Induction Strategies of Membrane Proteins in *Escherichia coli*

- choice of promoter and expression strains
- growth conditions
  - expression strains
  - growth medium
  - temperature
  - induction strategies (inclusion bodies?)

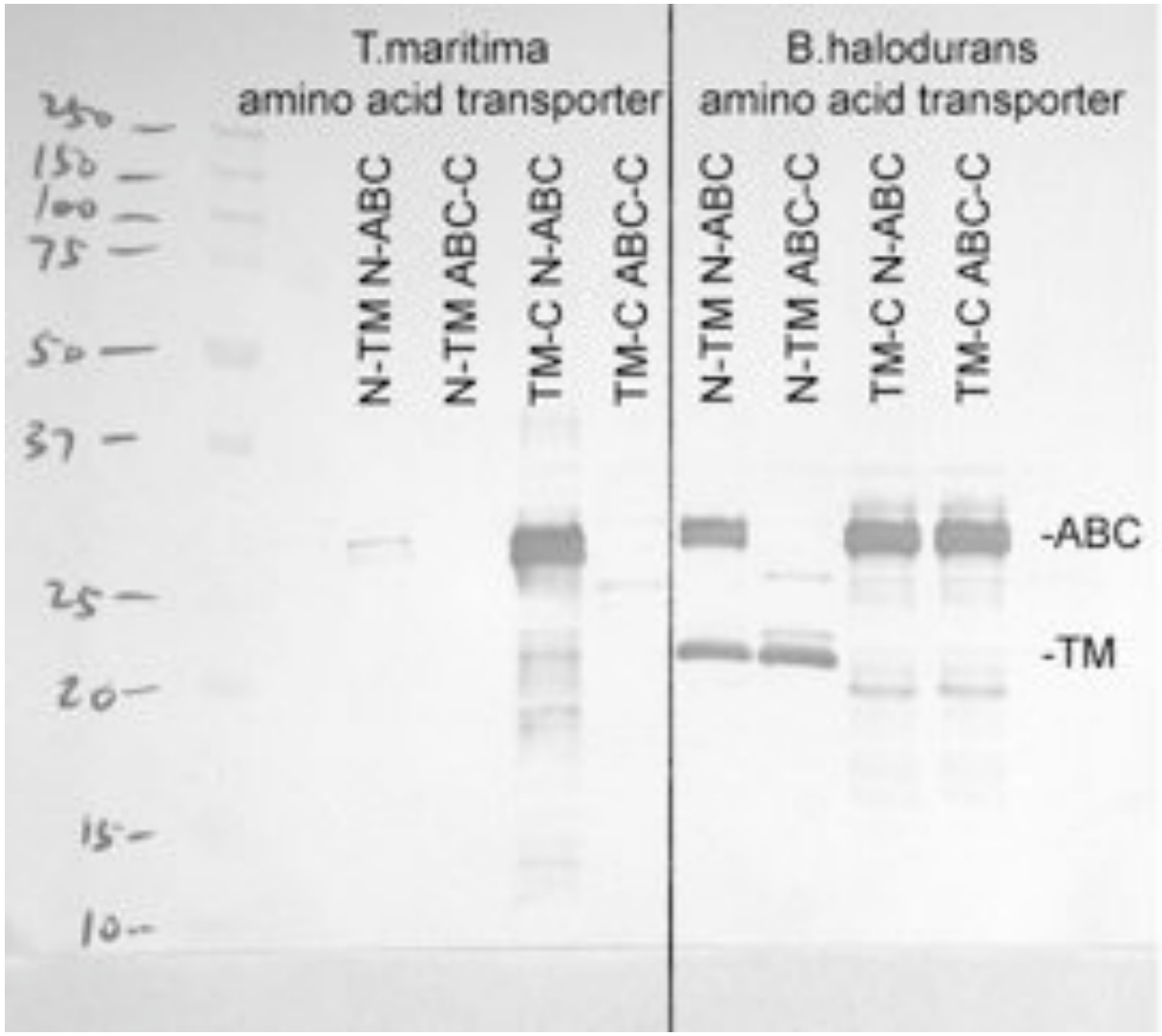
Media Screen

- Compare expression in Luria Broth (LB), Terrific Broth (TB), with additives (glucose, amino acids, etc.)
- Custom Media based on TB where we vary the extracts used and combination of extracts:
  - Yeast extract
  - Beef extract
  - Malt extract
  - Combination of extracts

affinity tags: no rules or guidelines

western blots of different his-tagged constructs of ABC transporters

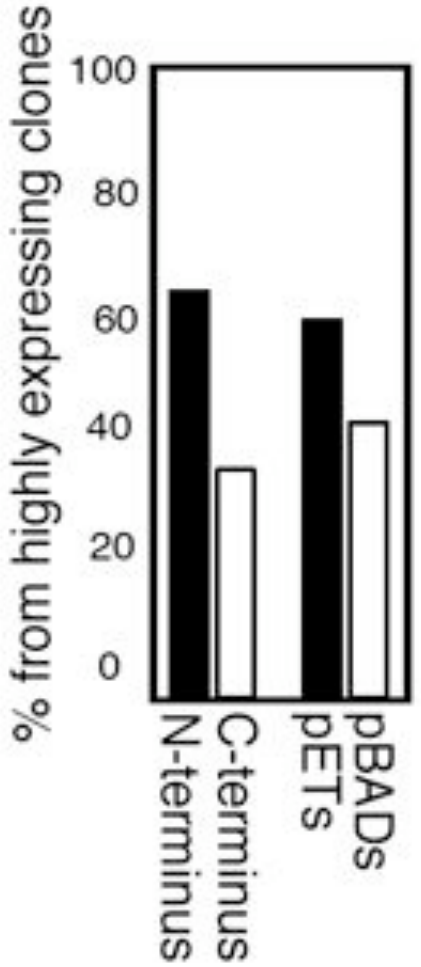
Zhenfeng Liu



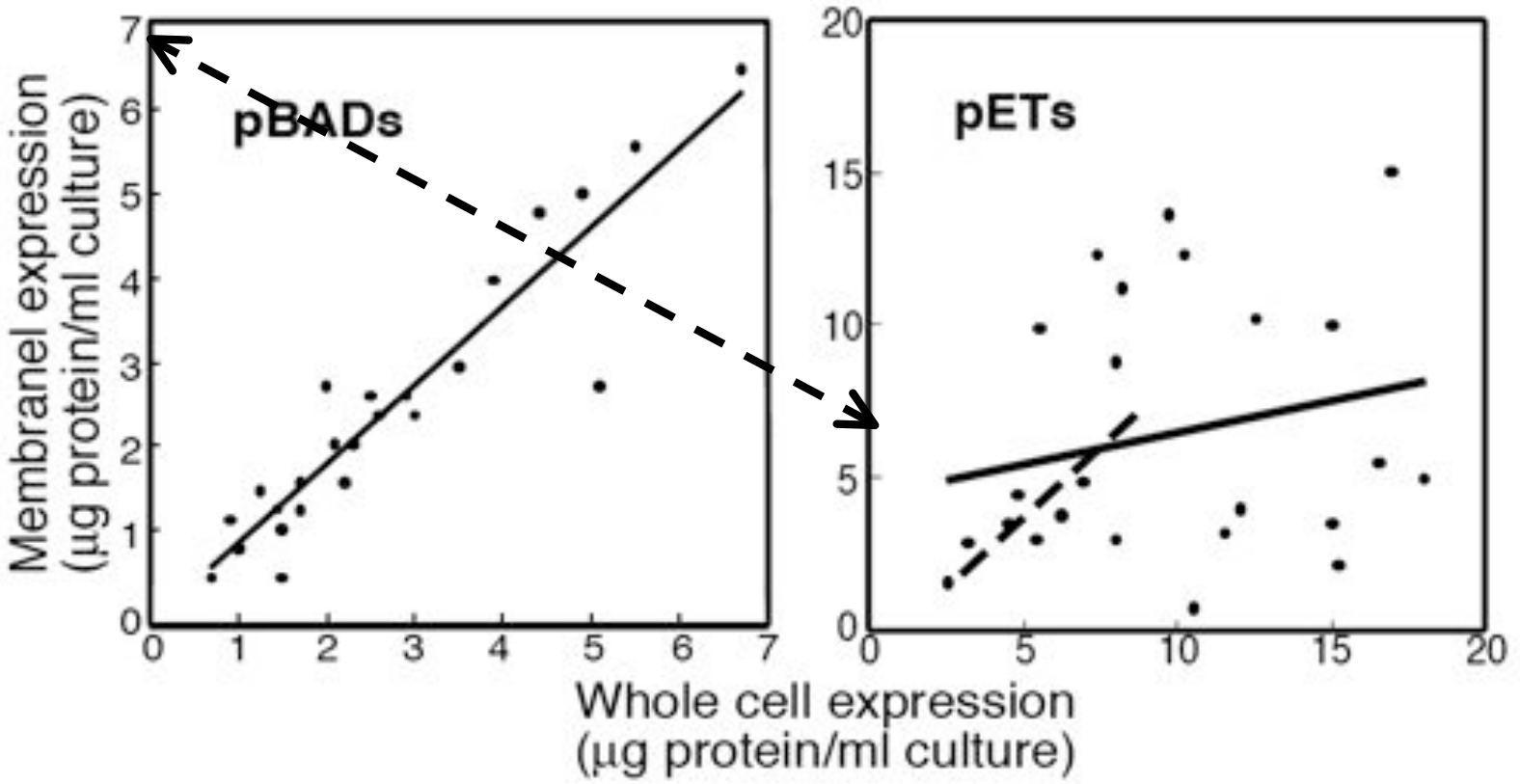
in general, we find N-terminal his-tags tend to express better than C-terminal his-tags

expression tests of prokaryotic P-type ATPases

Oded Lewinson

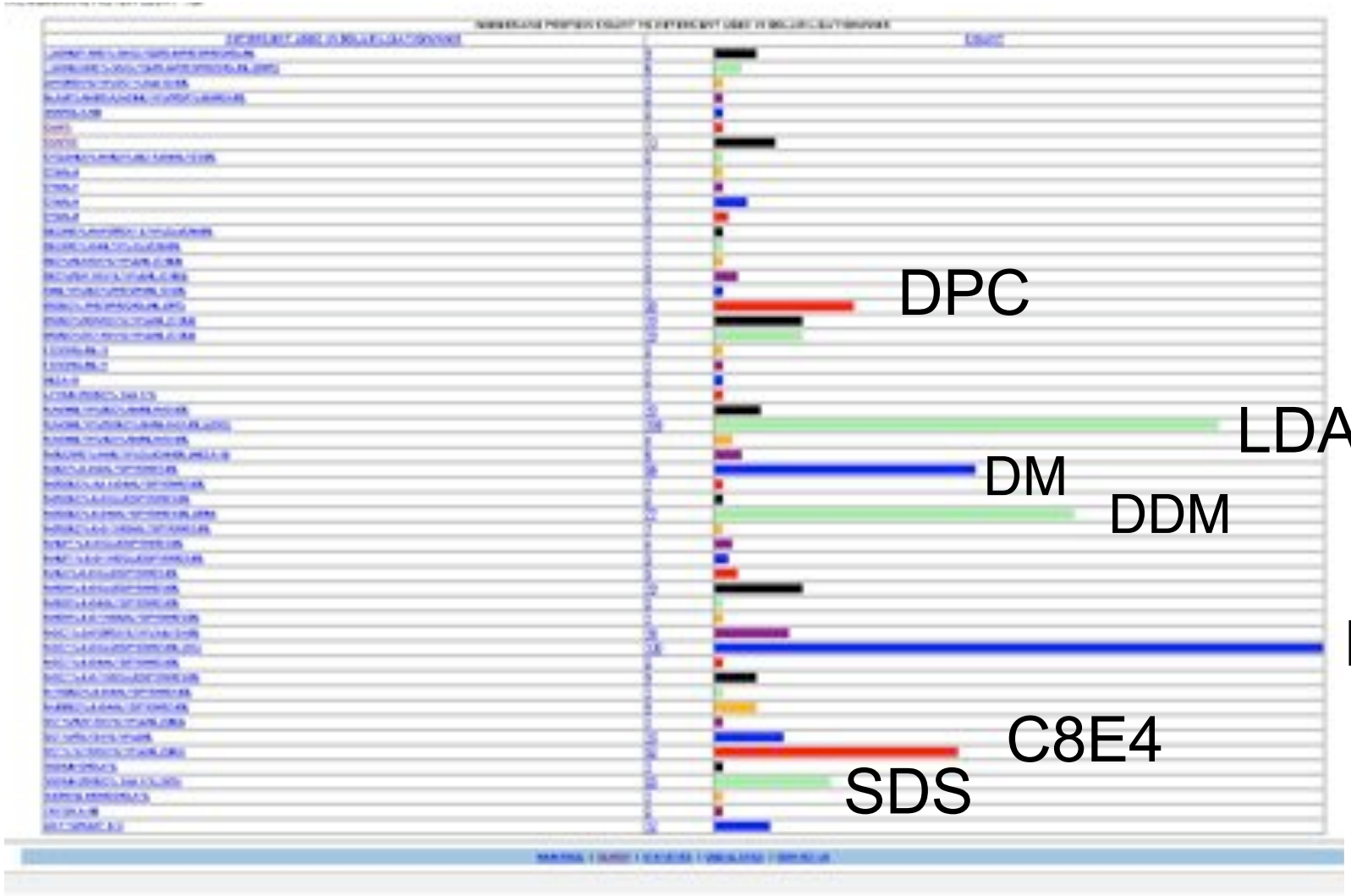


Whole cell expression levels may not reflect protein levels in the membrane



O. Lewinson

detergent extraction: some work better than others



fos-choline - friend or foe??

http://www.mpdb.ul.ie/

if at first you don’t succeed, try, try, again….

try something other than “old”detergents

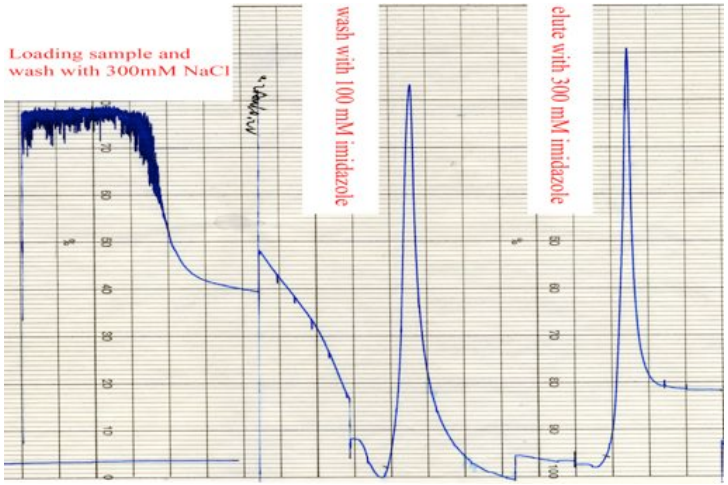
lipidic cubic phase, bicelles, “new” detergents

Protein Purification Considerations

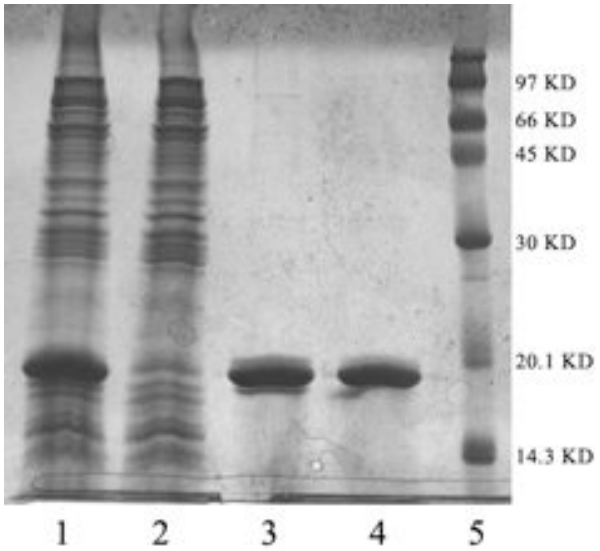
- Purification should be done expeditiously
- Lipid content will change during purification and ideally should be monitored
- Monodispersity of purified protein sample is prerequisite for crystallization, which can be checked by gel filtration, dynamic light scattering or EM.
- Use of concentrators with MWCO smaller than detergent micelle may lead to extremely high detergent concentration in the final protein sample.

Purification of Eco-MscL (Z. Liu)

- Wash column with 120 ml high salt buffer (20 mM Tris-HCl pH7.5, 300 mM NaCl, 30 mM imidazole, 0.05 % β-DDM) to remove non-specifically bound contaminants.
- Wash column with 100 mM imidazole buffer to remove weakly bound contaminants. Part of MscL proteins will elute at this stage.
- Elute MscL with 300 mM imidazole buffer, pool eluate and concentrate with 50 KD MWCO concentrator to 10-20 mg/ml protein.

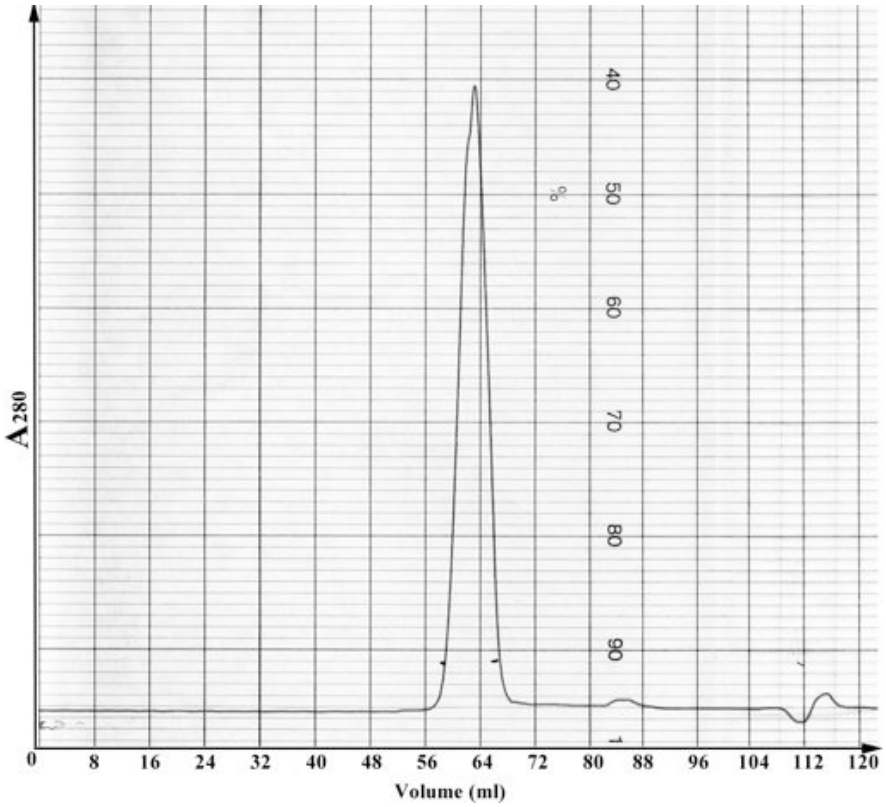


Ni-NTA chromatography

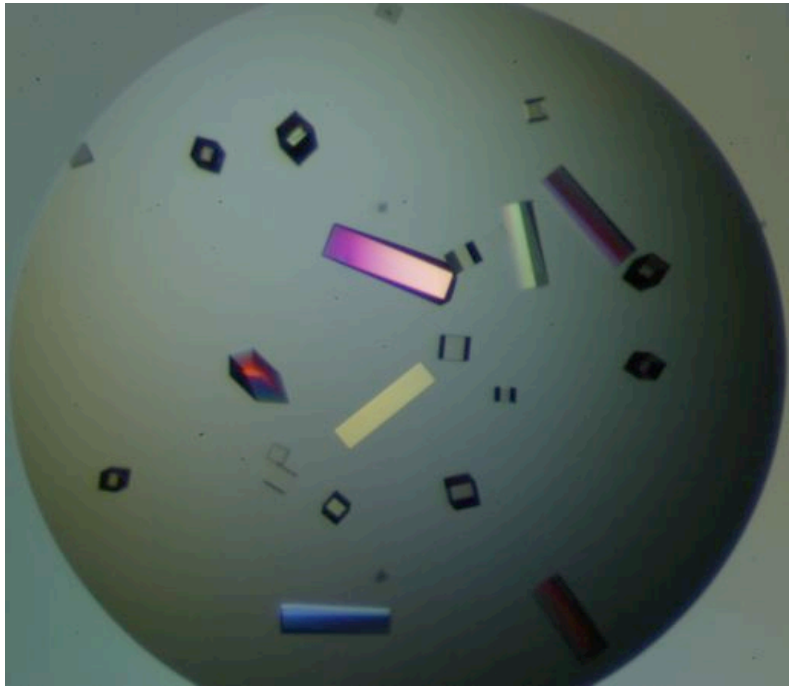


SDS-PAGE. Lane1, whole membrane extraction. Lane2, flow through. Lane3, 100 mM imidazole eluate. Lane4, 300 mM imidazole eluate. Lane 5, molecular weight marker.

Purification of EcMscL



E131A EcMscL in DDM
Superdex 200

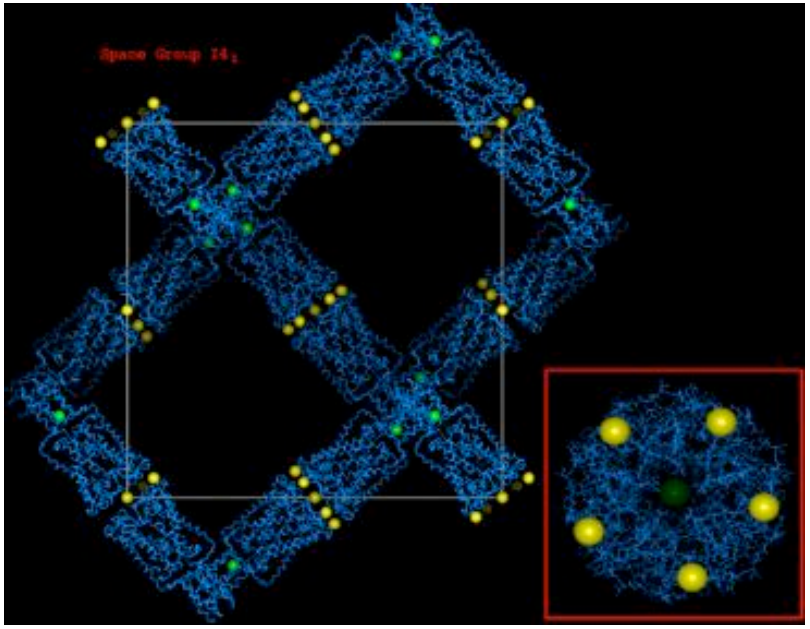


and crystallization

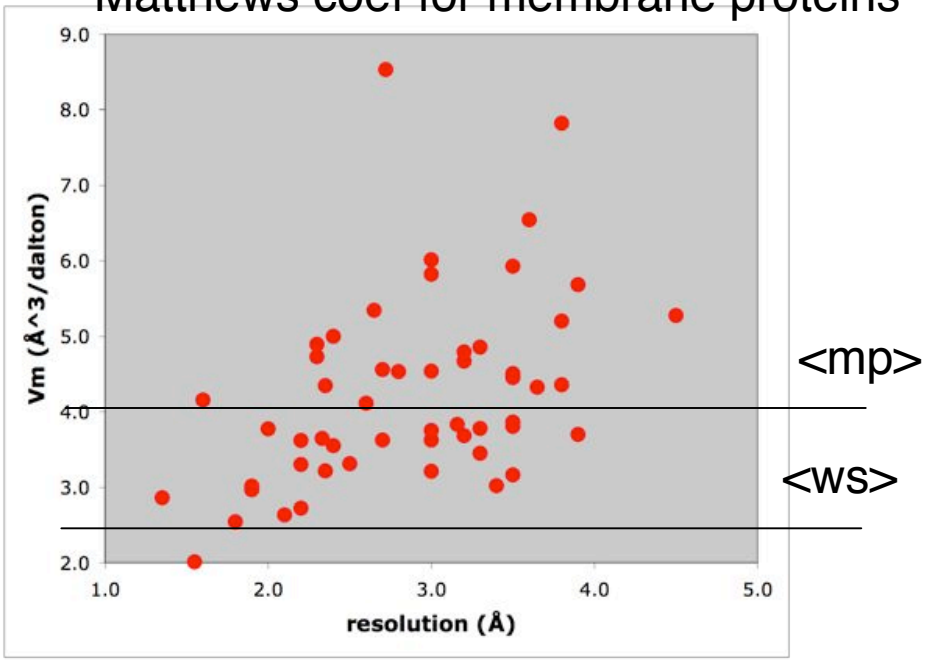
anisotropic diffraction between 7 - 4.5 Å resolution…

Membrane protein crystals

relatively high solvent content and modest diffraction quality



Matthews coef for membrane proteins



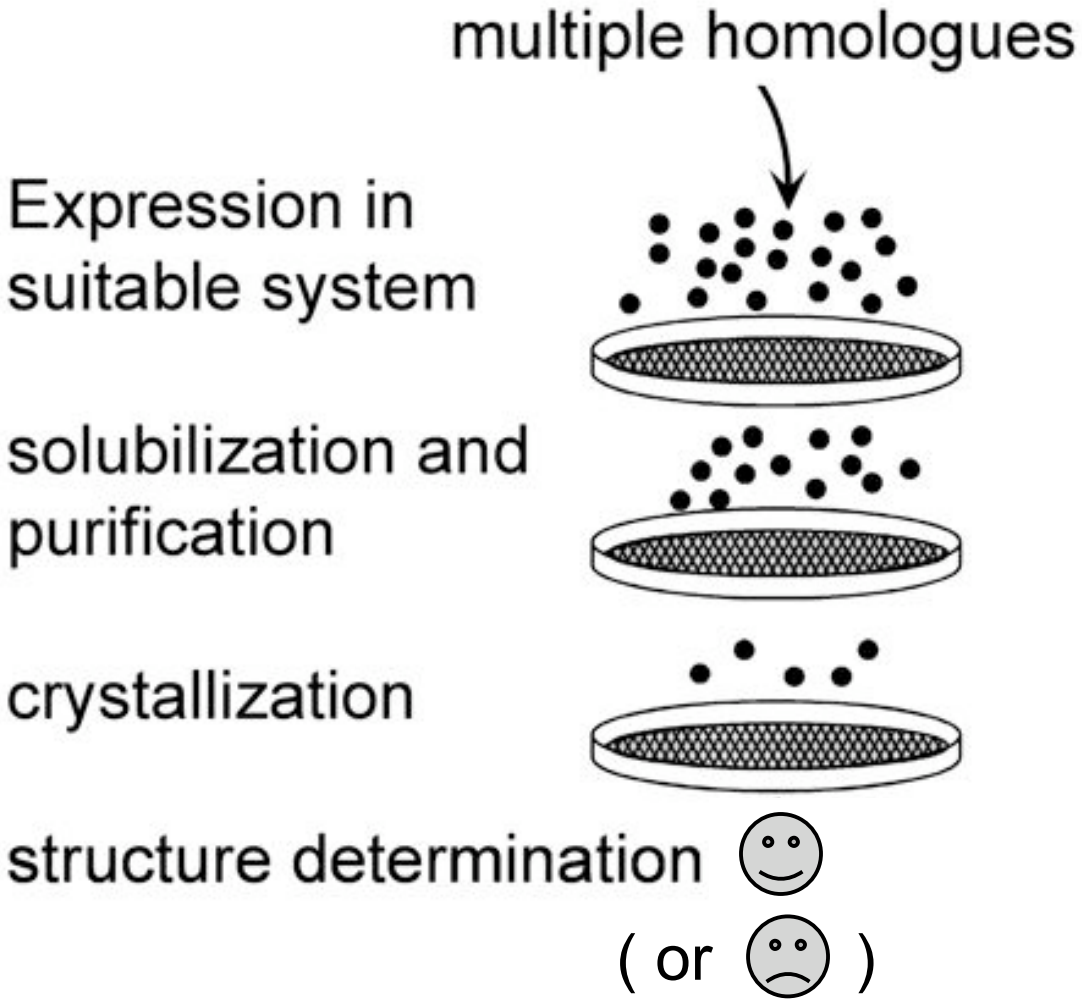
improving membrane protein crystals

- improving protein quality (purity, monodispersity and function)
- refining crystallization conditions
- addition of phospholipids (overpurification?), additives, ligands
- modifying constructs (tags, limited proteolysis, mutagenesis (surface))
- more homologues
- annealing/dehydration of crystals

state of the art crystallization robot at Caltech

Allen Lee

The funnel approach



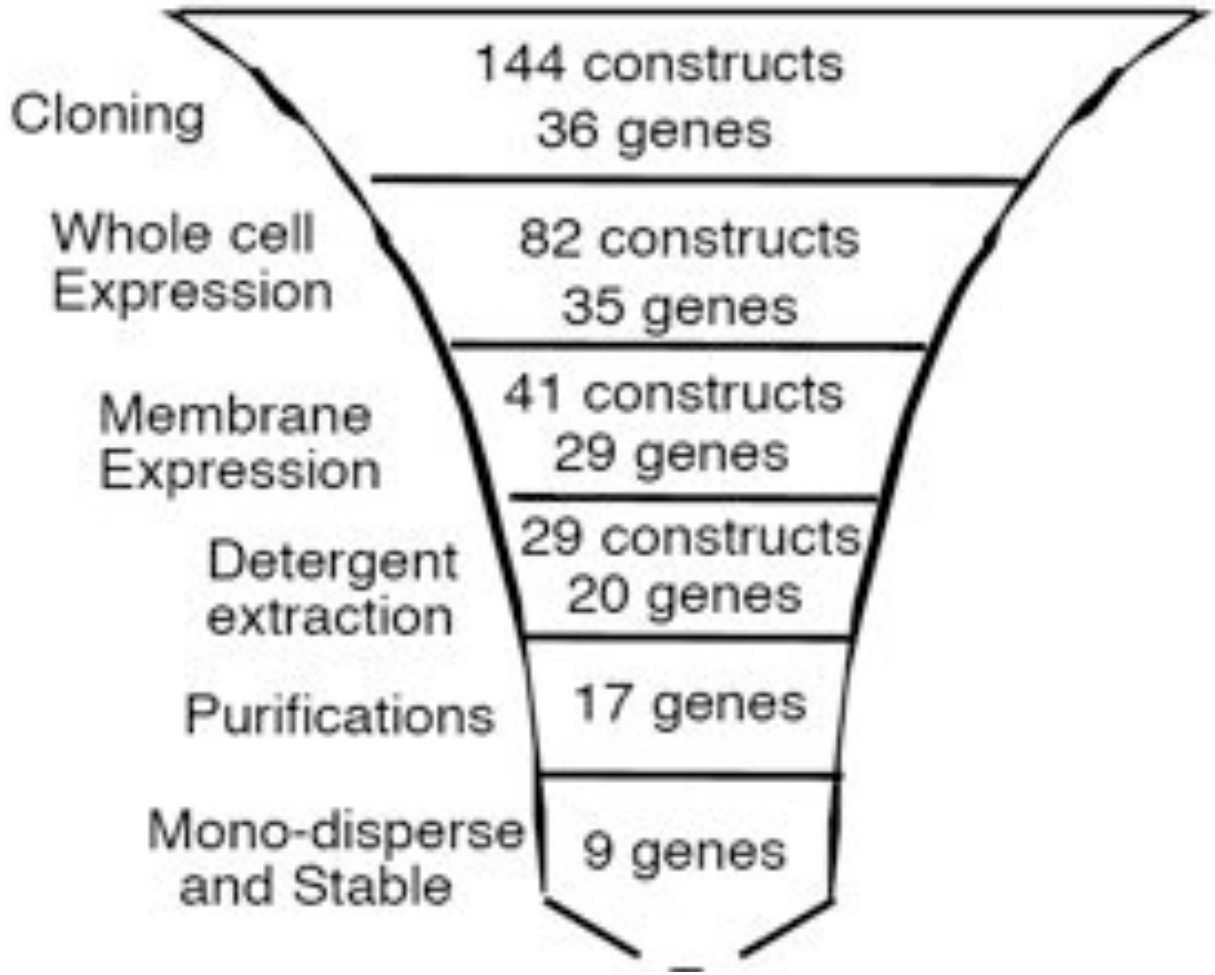
ABC transporters

original Locher screen
~26 transporters and 100 constructs
6 good expressors
3 structures (BtuCD, HI1470/71;
and *A. fulgidus* CysAT (Locher, ModABC))

P. Lum survey of molybdate transporters
~15 transporters - no good expression

Prokaryotic P-type ATPases

high-throughput screening



crystallization?? O. Lewinson

Conclusion

Happy membrane proteins are all alike;
every unhappy membrane protein
is unhappy in its own way.
(paraphrased from *Anna Karenin*, L. Tolstoy (1917))

It all depends on what protein you're talking about.
They're all different.
(paraphrased from H.R. Kaback (2007))

Structure of Proteins*

membrane

By Prof. J. D. Bernal, F.R.S.

THE structure of proteins is the major unsolved problem on the boundary of chemistry and biology to-day. We have not yet found the key to the problem, but in recent years a mass of new evidence and new lines of attack have enabled us to see it in a far more concrete and precise form, and to have some hope that we are near to solving it.

The problem of protein structure is twofold: the first is that of the form and properties of the protein molecule, and the second that of its internal structure. Owing to the extreme instability of the protein molecule, only the gentlest physical methods can be used; nevertheless three lines of attack—centrifugal, electrical, and X-ray—have already led to great success. The most fundamental has been the ultra-centrifuge, particularly the work of Svedberg and his school[1]. Protein

Bernal, *Nature* **143**, 663-7 (1939)

The picture thus presented is far from being a finished or even a satisfactory one. The crucial fact that requires elucidation is the precise mode of folding or coiling of the peptide chains, and for this we may have to wait for some considerable time, until the technique of X-ray and other methods have been advanced much further than at present. The problem of the protein structure is now a definite and not unattainable goal, but for success it requires a degree of collaboration between research workers which has not yet been reached. Most of the work on proteins at present is uncoordinated; different workers examine different proteins by different techniques, whereas a concentrated and planned attack would probably save much effort which is now wasted, and lead to an immediate clarifying of the problem.

[1] Svedberg, T., *Proc. Roy. Soc.*, A, 170, 40 (1939).
[2] Bernal, J. D., and Crowfoot, D., NATURE, 133, 794 (1934).
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[8] Personal communication.
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[14] Langmuir, I., and Wrinch, D., NATURE, 142, 49 (1938).

NIH Roadmap!

THANKS!!

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