Searching for Silver Bullets
An Alternative Strategy for Crystallizing Macromolecules

Bob Cudney[1] and Alexander McPherson[2]

[1]Hampton Research, Aliso Viejo, California [2]University of California Irvine, Irvine, California

What we did

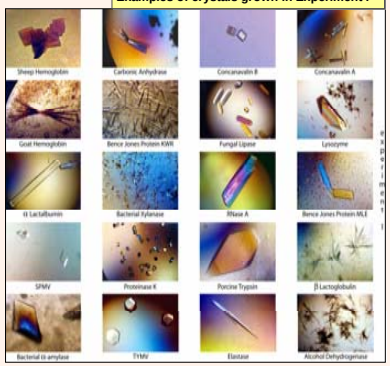
Based on a hypothesis that various small molecules might establish stabilizing, intermolecular, non covalent crosslinks in protein crystals and thereby promote lattice formation, we carried out three separate experiments. We assessed the impact of 200 chemicals on the propensity of 81 different proteins and viruses to crystallize. The experiments were comprised of 18,240 vapor diffusion trials. A salient feature of the experiments was that, aside from the inclusion of the reagent mixes, only **two** base crystallization conditions were used, 30% PEG 3350, and 50% Tacsimate™, both buffered at pH 7.

How we did it

- All reagents were:
  - Manually dispensed sitting drop vapor diffusion
  - Incubated at 25° Celsius
  - Reviewed manually at 20 and 200x using polarized light at 4, 8 and 12 weeks
  - Expt I used 3 different reservoir concentrations, Expt II used 2, and Expt III used 1
- All experiments used two fundamental conditions:
  30% w/v PEG 3,350, 0.1 M Hepes pH 7.0
  50% Tacsimate™ pH 7.0
- A total of 200 chemicals compounds were tested.
- Screened a total of 81 total proteins in all 3 expts: Expt I=60; Expt II=67; Expt III=66
- Expt I & II used 48 different reagent mixes, 2 of which were PEG and Tacsimate controls. The remaining 46 reagents were comprised of 3 to 20 different chemicals, on average 3 to 6 chemicals in each reagent.
- Expt III used 24 different bioactive compound reagent mixes, 1 of which was a PEG control. Each reagent is comprised of 4 to 5 bioactive compounds such as coenzymes, prosthetic groups, inhibitors, drugs, effectors, nucleotides, amino acids/peptides, and sugars/oligosaccharides. Sample arrays set in duplicate.
- A total of 18,240 experiment drops were set for three groups of experiments.

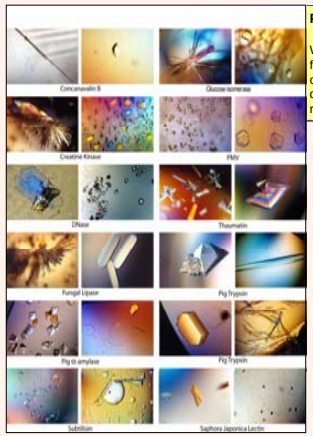
Experimental Results

Examples of crystals grown in Experiment I

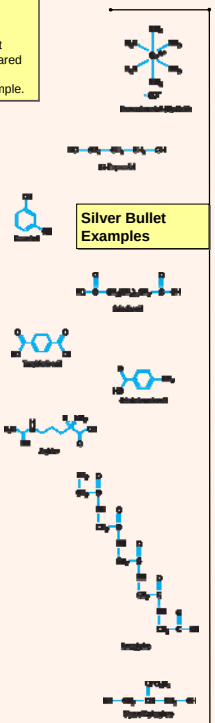


Polymorphisms

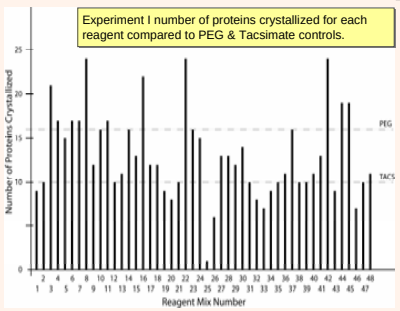
With considerable frequency, different crystal forms appeared depending on the reagents in the sample.

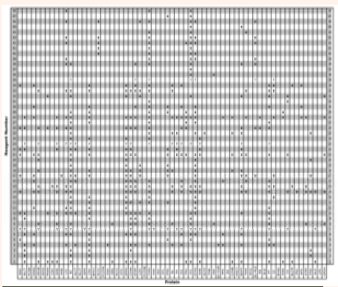


Silver Bullet Examples



Experiment I number of proteins crystallized for each reagent compared to PEG & Tacsimate controls.





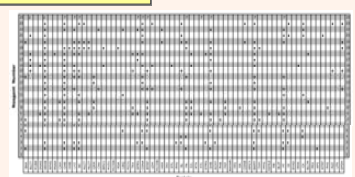
Expt II ◆ Crystals T Tacsimate control P PEG control

Experiment I
1. spermidine, spermine, cadaverine, putrascine, 1,8 diamino octane
2. 6-amino hexanoic acid, 3-aminopropionic acid, 4-aminobutyric acid
3. oxamic acid, salicylic acid, 4-aminobenzoic acid
4. Mix #2 and Mix #3
5. glutaric acid, hexadecanoic acid, dodecanedioic acid, fumaric acid
6. oxalic acid, maleic acid, oxalacetic acid, terephthalic acid
7. Mix #5 and Mix #6
8. Pentaglycine, triglycine
9. poly-L-lysine, poly-L-ornithine, poly-DL-alanine
10. poly-L-glutamic acid, poly-DL-alanine
11. Mix #9 and Mix #10
12. Protamine
13. heparin, dextran sulfate
14. d(pA)4, d(pA)6
15. dp(T)4, d(pT)12
16. pyrophosphate, tetrapolyphosphate
17. ornithine, arginine, asparagines, glutamine
18. Tacsimate, PEG 3350
19. glycerol, sucrose, sorbitol
20. TMAO, proline
21. sarcosine, glutamate, glycine, betaine
22. transaconitic acid, glyceric acid, indolbutyric acid, D-hydroxyphenylacetic acid
23. palmitic acid, octanoic acid, sterylamine
24. barbituric acid, resorcinol, glycerol-2-phosphate
25. praseodymium acetate, gadolinium chloride, cobalt hexamine
26. hexadecyl trimethylammonium bromide
27. Tacsimate, Mix #8
28. Tacsimate, poly-L-ornithine, lysine, glutamic acid, alanine
29. Tacsimate, Mix #2
30. Tacsimate, Mix #3
31. Tacsimate, Mix #5
32. Tacsimate, Mix #5
33. Tacsimate, Mix #1
34. Tacsimate, Mix #19
35. Tacsimate, Mix #17
36. Tacsimate, Mix #13
37. PEG 3350
38. Tacsimate, d(pA)4, d(pA)6
39. Tacsimate
40. Tacsimate, d(pT)12, d(pT)4
41. Mix #8, Mix #9
42. Mix #8, Mix #9
43. Mix #1, Mix #5
44. Mix #1, Mix #3
45. The 20 amino acids
46. Tacsimate, the 20 amino acids
47. Tacsimate, the 20 amino acids
48. Tacsimate, protamine

Reagent Mix Expt I

Experiment II
1. schardinger's dextrin
2. NDSB-201
3. NDSB-195, NDSB-201, NDSB-211, NDSB-221, NDSB-256
4. tannic acid
5. streptomycin
6. trimesic acid, phloroglucinol
7. tetraphenylporphorin, cobalt hexamine
8. mellitic acid, myo-inositol, phytic acid
9. Mix #8 and Mix #8
10. piperizine ethane sulfonic acid, anthraquinone disulfonic acid
11. resourcinol, tetrahydroxybenzoquinone
12. pyromellitic acid, 2,2-thiodiglycolic acid, barbituric acid, terephthalic acid
13. fumaric acid, maleic acid, glutaric acid, pimelic acid, suberic acid
14. suberic acid, sebacic acid, hexadecanedioic acid, dodecanedioic acid
15. fumaric acid, pimelic acid, sebacic acid, hexadecanedioic acid
16. maleic acid, glutaric acid, suberic acid, sebacic acid, dodecanedioic acid, oxamic acid
17. trehalose, sucrose, melibiose, maltose, cellobiose
18. raffinose, maltotriose, melizitose
19. melibiose, stachyose
20. cyclodextrin, 2-hydroxypropyl-β-cyclodextrin, 2,6-di-O-methyl-β-cyclodextrin
21. di, tri, tetra, pentaglycine
22. 1,3-propanediol, 1,2-butanediol, MPD, 1,6-hexanediol
23. PEG 3350
24. fumaramide, 1,4-diaminobutane, spermine, 1,8-diaminooctane, cadaverine
25. pyrophosphate, phosphorous acid triethyl ester, phytic acid
26. Mix #4 and Mix #10
27. MDSB-201 and Mix #13
28. Mix #15 and Mix #16
29. phenyl urea, sodium-1-pentanesulfonate, sulfanilic acid, salicin, pentanetricarboxylic acid
30. oxamic acid, fumarate, fumaramide, putracine, pentane tricarboxylic acid
31. tranaconitic acid, PABA, sulfanilic acid, 3-indolebutyric acid, pentane tricaroxylic acid
32. β-thiocyliglucoside, Anapoe-305, MEGA-9, nonyl-β-D-glucoside
33. trimesic acid, mellitic acid, pyromellitic acid, terephtalic acid
34. phloroglucinol, phytic acid, anthroquinone disulfonic acid, barbituric acid, tetraphenyporphorin
35. Tacsimate
36. Tacsimate and Mix #2
37. Tacsimate and Mix #6
38. Tacsimate and Mix #8
39. Tacsimate and Mix #10
40. Tacsimate and Mix #12
41. Tacsimate and Mix #13
42. Tacsimate and Mix #14
43. Tacsimate and Mix #15
44. Tacsimate and Mix #19
45. Tacsimate and Mix #20
46. Tacsimate and Mix #26
47. Tacsimate and Mix #24
48. Tacsimate and Mix #21

Reagent Mix Expt II



Expt III ◆ Crystals P PEG control

Experiment III – Bioactive Compounds
1. s-adenosyl-methionine, β- glycerophosphate, stachyose
2. biotin, phosphoenol, pyruvate, melizitose
3. FAD, phosphoglyceric acid, spermine
4. thymine, pyrophosphate, glyceric acid, Schardinger's dextrin
5. NAD, fructose-1,6-diphosphate, spermidine
6. pyridoxyl-5'-phosphate, pyruvic acid, cyclodextrin
7. ADP, AMP, UMP
8. dAMP, GDP, naladixic acid
9. GTP, dTMP, chloropromazine
10. ATP, dCMP, estradiol, galactose
11. Tacsimate
12. lipoic acid, creatine, glutathione, pantothenic acid
13. nicotinamide, batyl alcohol, glutaric acid
14. flavin mononucleotide, triolein, acetyl choline
15. pepstatin A, GMP, fumaric acid, glucose
16. NADP, tetrahydrolic acid, ascorbic acid, glucose
17. riboflavin, phosphatidyl choline, raffinose
18. dGMP, cholesterol, thymine, oxamic acid
19. phosphocreatine, benzoyl-arginine ethyl ester, phenobarbital, tetracycline
20. UTP, karmycin, maltotriose, leupeptin
21. phosphorylribose-1-pyrophosphate, maleic acid, n-acetyl-D-glucosamine
22. 3'-UMP, ribose, phytic acid, palmitic acid
23. The 20 amino acids
24. PEG 3350

Reagent Mix Expt III

Positive charge centers, Diols, Dicarboxylic acids, Mixed amino and carboxylic acids, Polar amino acids, Glycine oligomers, Organic acids, Charge symmetric, and Diamines

Condensation of Statistics

	Experiment All	I	II	III
How many proteins were investigated?	81	60	67	66
How many proteins crystallized?	65	48	50	50
How many proteins crystallized				
in Polyethylene glycol controls?	20	11	14	13
in Tacsimate controls?	6	5	4	N/A
in both controls?	5	5	3	N/A
Total controls	31	21	21	13
How many proteins crystallized only in the presence of some reagent mix?	35	28	29	37
How many reagents exceeded the				
Polyethylene glycol controls?		11	7	4
Tacsimate controls?		7	1	N/A
How many proteins that did NOT crystallize in controls, crystallized in ONLY				
One reagent mix?		4	7	13
Two reagent mixes?		2	4	9
Three reagent mixes?		1	4	6

What happened

Overall, 65 proteins (85%) were crystallized. Most significant was that 35 of the 65 (54%) crystallized only in the presence of one or more reagent mixes, but not in control samples lacking any additives. Among the most promising types of reagent mixes were those composed of polyvalent, charged groups, such as di and tri carboxylic acids, diamino compounds, molecules bearing one or more sulfonyl or phosphate groups, and a broad range of common biochemicals, coenzymes, biological effectors, and ligands. Less promising reagent mixes were composed of osmolites, polyamines, detergents and sugars.

Summary

We propose that an alternate approach to crystallizing proteins might be developed, which employs a limited set of fundamental crystallization conditions combined with a broad screen of potentially useful small molecule additives.

What's next

The evaluation of more reagent mixes, the influence of pH and the use of common dehydrants.

Thank you Aaron Greenwood, Joe Luft & Peter Nguyen.