Crystal cookery – using high-throughput technologies and the grocery store as a teaching tool

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### Undergraduate Research Experiences

The Hauptman-Woodward Medical Research institute has been running an undergraduate summer intern program sponsored by local organizations for over a decade.

Students are paid a stipend.

Students spend **10** weeks conducting individual research projects in PI laboratories. Each research project is related to the PI's research.

At the end of these 10 weeks, each student presents an overview of the project and their research to the institute staff and their peers.

Depending on the year numbers can range from 10-20.

Information is available at http://www.hwi.buffalo.edu/outreach/internships.html

### Why use crystals as a 'hook' for this?

They are stunningly beautiful. They link biology to many other disciplines. For some proteins, it's not hard to get them.

# The Scientific Method

#### THE SCIENTIFIC METHOD ... FOR TEN-YEAR OLDS



\* THIS IS SUPPRISINGLY CLOSE TO HOW REAL SCIENTISTS ACT AT CONFERENCES.

http://www.sheldoncomics.com/archive/100806.html

### **Practical Considerations**

### Practical Considerations

#### Logistics

- Where do I sit?
- Where are the supplies?

#### Time

- Get to a point where you have time to analyze results and make conclusions
- Ensure there is time to prepare a presentation
- Plan the experiment so that the first few days are getting used to the environment
- In reality, a 10 week program is 8 weeks of solid work for a new entry

Interest

 A student is not a pipetting robot (which are more expensive but really cool)

## 1. Define the question

### Define the question (my motivation)

Undergraduate students add a dynamic component to the lab and it's gratifying to be influential at this point of a potential new scientists career.

I have a group of new undergraduate summer students coming into the lab for 10 weeks (I couldn't say no). I have limited information on their expertise, I don't know how they will interact with the rest of the laboratory.

I have preliminary data on these but require experimental proof.

I need safe projects to test their expertise, one that identifies strengths and weaknesses, allows me some time to do my research, and one where they benefit and enjoy the experience.

### Define the question (from a science viewpoint)

Science:

Crystallization – Chemistry, Physics and Biology

Structural biology – Medicine, Pharmacy, Energy, Agriculture, Veterinary studies etc.

Lots of hooks.

Silver Bullets are a carefully researched and designed library of compounds with the potential and ability to promote crystallization.

Each Silver Bullet solution contains between 2 and 20 small molecules.

The solutions use low concentrations of these small molecules.

Searching for silver bullets: An alternative strategy for crystallizing macromolecules. Alexander McPherson and Bob Cudney. Journal of Structural Biology 156 (2006) 387-406.

A novel strategy for the crystallization of proteins: X-ray diffraction validation. Steven B. Larson, John S. Day, Robert Cudney, and Alexander McPherson. Acta Cryst. (2007) D63, 310-318.

### 2. Gather information and resources

Gather Information and Resources (set questions)

What are Silver Bullets?

Explore the commercial and scientific literature. Introduce citation searching

How do they work?

Understand the literature, ask questions, communication in the laboratory.

What chemicals promote crystallization?

# 3. Form a hypothesis

### Form a hypothesis (based on guidance)

What are the proteins?

What is their function?

What chemicals might interact with them?

What might promote crystallization?

What grocery store items might influence crystallization ?

Choose Proteins (20 in our case)

	Protein name	Concentration (mg ml <sup>-1</sup> )	MW (kDa)	pI†	Source	Manufacturer‡
Α	Ubiquitin	10	9	6.8 (E)	Bovine erythrocytes	Sigma U6253
в	Cytochrome c	10	12	9.6 (E)	Bovine heart	Sigma C2037
С	Lysozyme	50	14	10.7 (E)	Chicken egg white	Sigma L7651
D	$\beta$ -Lactoglobulin A	10	18	5.1 (E)	Bovine milk	Sigma L7880
Е	Thaumatin	10	21	12 (E)	Thaumatococcus daniellii	Sigma T7638
F	Papain	31	23	9.3 (E)	Papaya	Sigma P4762
G	Chymotrypsinogen A	10	25	9.1 (C)	Bovine liver	Worthington LS005630
Н	Chymotrypsin	10	26	8.3 (E)	Bovine liver	Worthington LS001448
Ι	Lipase	10	27	4.4 (E)	Aspergillus niger	Sigma 84205
J	Proteinase K	10	27	8.9 (E)	Tritirachium album limber	Worthington LS004222
к	Ovalbumin	20	44	5.1 (E)	Chicken egg white	Sigma A5503
L	Hemoglobin	20	64	7.1 (E)	Bovine erythrocytes	Worthington LS00242
Μ	Hemoglobin	10	67	8.1 (E)	Horse erythrocytes	Sigma H4632
Ν	Hemocyanin	4.5	73	5.9 (T)	Limulus polyphernus	Sigma H1757
0	Creatine kinase	10	81	7.4 (E)	Rabbit muscle	Sigma C3755
Р	Lactoferrin	10	90	8 (E)	Bovine milk	Sigma L9507
Q	Concanavalin A	10	102	8.35 (E)	Jack bean (Canavalia ensiformis)	Sigma L7547
R	Alcohol dehydrogenase	10	141	6.91 (C)	Yeast (Saccharomyces cerevisiae)	Worthington LS001069
S	Catalase	16	240	5.5 (E)	Bovine liver	Sigma C30
Т	Urease	10	480	4.9 (E)	Jack bean (Canavalia ensiformis)	Worthington LS003887

Proteins used in this work, listed with molecular weights (MW) and experimental (where available) or calculated pI.

† For pI, T = theoretical (calculated), C = catalog value and E = experimental data. \$\\$Worthington. The catalogue number is listed after the manufacturer information.

Available commercially in large quantities at reasonable cost

Choose Chemicals (96 in our case)

### Our Chemical Stock Room

Note:

 The lack of safety equipment
 No MSDS's
 Presence of non-scientific staff
 The most expensive 'chemical' is well under \$10
 Everything is available in 'large' quantities
 No expensive lab equipment

	Number	Name	Ingredients, where available
	1	Bubbles	0.5 cup dish soan. 1.5 cups water, 2 teaspoons sugar
	2	White vinegar	Select sun-ripened grain, diluted with water
	3	Clorox kitchen cleaner	Not available
	5	White grape juice Dove Weightless Moisturizers hairspray with natural movement – extra hold	White grape juice, citric acid, accorbic acid, potassium metabiculite SD alcohol 40-b alcohol denatured, dimethyl ether, hydrofluorocarbon 152A, VA/crotonates/vinyl neodecanoate copolymer, acrylates/hydroxyesters acrylates copolymer, aminomethyl propanol, fragrance (parfum), sodium benzoate, cyclohexylamine, dimethicone copolyol, cyclopentasiloxane, silk amino acids, alanine, glycine, serine, arginine, isoleucine, cystine, histidine, glutamic acid, lysine, <i>Borago officianilis</i> seed oil ( <i>Borago officinalis</i> ), palmitic acid, stearic acid, linoleic acid, oleic acid, eicceenoefic acid, elywerin
	6	Scope mouthwash	Alcohol, glycerin, flavor, polysorbate 80, sodium saccharin, sodium benzoate, œtylpyridinium chloride,
	7	Nyquil liquid, cherry flavored	benzoic acid, Blue 1, Yellow 5 Acetaminophen, dextromethorphan HBr, doxylamine succinate, alcohol, citric acid, FD&C Blue No. 1, FD&C Red No. 40, flavor, high-fructose corn syrup, polyethylene glycol, propylene glycol, purified water, saccharin sodium citrate
	8	Proactive Solution revitalizing toner	Glycolic acid, Hamanelis virginiana (witch hazel) extract, Anthemis nobilis flower extract, Rosa canina fruit extract, Aloe barbadensis leaf juice, sodium PCA, panthenol, glycerin, propylene glycol, allantoin, polysorbate 20, hydroxyethylcellulose, sodium hydroxide, benzophenone-4, tetrasodium EDTA, imidazolidinyl urea, methylparaben, propylparaben, fragrance (parfum), Blue 1 (CI 42090), Vellow 5 (CI 19140)
	9	Sudafed PE children's nasal decongestant	Phenylephrine HCI, citric acid, edetate disodium, FD&C Red No. 40, flavor, glycerin, sodium benzoate, sodium carboxymethyleellulese sodium citrate sorbiol sucralese
	10 11	Red wine vinegar Coca Cola, Diet	Red wine vinegar (reduced with water to 5% acidity), potassium metabisulfite Carbonated water, caramel (color), aspartame, phosphoric acid, potassium benzoate, natural flavors, citric acid, caffeine
	12	Listerine anticavity fluoride rinse	Sodium fluoride, sorbitol solution, alcohol (21.6%), poloxamer 407, sodium lauryl sulfate, phosphoric acid sucralose dibasic sodium phosphate. D&C Red No. 33. ED&C Blue No. 1
	13	Kosher dill spears juice	Vinegar, salt, calcium chloride, natural flavor, garlic oil, polysorbate 80, Yellow 5, Blue 1
	14	Sparkling beverage, diet, peach grapefruit	Carbonated water, citric acid, potassium citrate, natural flavor, aspartame, potassium benzoate (a preservative), acesulfame potassium
	15	Heinz All Natural cleaning vinegar	Not available
	16 17	Febreze fabric refresher Alcon Opti-Free disinfecting solution	Alcohol, odor eliminator derived from corn, fragrance Sodium chloride, sodium borate, propylene glycol, Tearglyde proprietary dual-action reconditioning system (Tetronic 1304, nonanoyl ethylenediaminetriacetic acid) with Polyquad (polyquaternium-1)
1	18	Hydrogen peroxide	0.001% and Aldox (myristamidopropyl dimethylamine) Stabilized hydrogen persovide 3%
	19	Fat-free milk	Fat-free injurges percent of the second seco
	20	Clean & Clear deep-cleaning astringent	Salicylic acid, alcohol (24.5%), algae extract, Alee barbadensis leaf extract, benzophenone-4, denatonium benzoate, dimethicone propyl PG-betaine, fragrance, glycerin, isoœteth-20, PEG 32, propylene glycol, sodium citrate, Blue 1
5		Dove Weightless Moisturi	zers hairspray with SD alcohol 40-b alcohol denatured, dimethyl ether, hydrofluorocarbon 152A, VA/crotonates/vinyl
		natural movement - ex	tra hold neodecanoate copolymer, acrylates/hydroxyesters acrylates copolymer, aminomethyl propanol,
			fragrance (parfum), sodium benzoate, cyclohexylamine, dimethicone copolyol, cyclopentasiloxane,
			silk amino acids, alanine, glycine, serine, arginine, isoleucine, cystine, histidine, glutamic acid, lysine,
			Borago officinalis seed oil (Borago officinalis), palmitic acid, stearic acid, linoleic acid, oleic acid,
			eicosenoic acid, glycerin
-			laureth-8 sulfate, sodium oleth sulfate, sodium methylparaben, DMDM hydantoin, magnesium
	28	Palmolive gel dishwasher detergent	iaureth-8, magnesium oleth suifate, polyquaternium-10, propylene glycol, disodium EDTA Phosphorus content: averages 1.6% phosphorous, in the form of phosphate, sodium hydroxide, sodium
	29	Purell instant hand sanitizer	Isopropyl alcohol, glycerin, carbomer, fragrance, aminomethyl propanol, propylene glycol, isopropyl
	30	Airborne baby wash	Not available
	31	Dawn Plus dishwashing liquid	Biodegradable surfactants (anionic and nonionic), enzymes
	32	Excedrin	Acetaminopnen, asprin, catteine, benzoic acid, carnauba wax, hydroxypropyi cellulose, hydroxypropyi methylcellulose, microcrystalline cellulose, mineral oil, polysorbate 20, povidone, propylene glycol, simethicone emulsion, sorbitan monolaurate, stearic acid, FD&C Blue 1, titanium dioxide
	33 34	Brach's Star Brites cinnamon mint candy Dish detergent, lemon-scented	Corn syrup, sugar, artificial and natural flavor, titanium dioxide color, Red 40, Red 3, Blue 1, Yellow 6 An aqueous solution of anionic and nonionic surfactants, stabilizers, perfume, colorant and quality control agents
	35	Arm & Hammer pure baking soda	Sodium bicarbonate

Number	Name	Ingredients, where available
36	Totally Light 2 Go cranberry pomegranate mix	Citric acid, ascorbic acid (vitamin C), artificial flavor, potassium and sodium citrate, acesulfame potassium, purified polyphenols, natural and artificial pomegranate flavor, sucralose, gum arabic, magnesium oxide. FD&C Red 40. tricalcium phosphate. FD&C Blue 1
37	Tilex mildew root penetrator and remover	Sodium hydroxide, sodium hypochlorite
38	Lemon juice	Lemon juice from concentrate, sodium benzoate, sodium metabisulfite and sodium sulfite, lemon oil
39	Maple extract	Pure maple syrup concentrate, alcohol, glycerin, sugar, caramel (color)
40	Afta aftershave lotion	Balamic Vinegar SD alsolid (J. DEC 2 staarsta PDC 2 myristyl ather proving to fragrance trigthanologija, carbomer
41	Arta artersnave forion	diazolidinyl urea, methylparaben, PEG 2 stearate SE, stearic acid, propylparaben, allantoin, BHT, diazolidinyl Urea, D&C Green No. 5. FD&C Yellow No. 5
42	Heinz tomato ketchup	Lycopene, tomato concentrate, distilled vinegar, high-fructose corn syrup, corn syrup, salt, spice, onion
43	Crystal Light raspherry lemonade mix	powder, natural flavors
45	Crystal Light faspoerty lemonade mix	alanine) (sweetener), raspberry juice solids, natrificial flavor, acesulfame potassium
1		(sweetener), soy lecithin, artificial color, Red 40, Blue 1, tocopherol
44	Ground pepper	Black pepper
45	Powder dishwasher detergent	Sodium carbonale, sodium silicate, enzymes
40	Salt jodized	Ground cinnanion
48	Avon Personal Match loose powder	Not available
49	Cascade automatic dishwasher detergent	Fragrance(s)/perfume(s), surfactants, sodium sulfate anhydrous, cleaning agents, colorant/pigment/dye, bleach, sudsing agent, water softeners (complex sodium phosphates/sodium carbonate)
42	Heinz tomato ketchup	Lycopene, tomato concentrate, distilled vinegar, high-fructose corn syrup, corn syrup, salt, spice, onion
		powder, natural flavors
32	supplement, tablets	cellulose, ascorbic acid (vitamin C), ferrous fumarate, ptalpha tocopheryl acetate (vitamin E),
		acacia, anhydrous citric acid, ascorbyl palmitate, beta carotene, biotin, boric acid, BHT, calcium
		pantothenate, calcium stearate, cholecalciferol (vitamin D), chromic picolinate, corn starch,
		crospovidone, cupric sulfate, cyanocobalamin (vitamin B12), pL-alpha tocopherol, FD&C Yellow 6
		Auminium Lake, tote acid, getatin, nydrogenaed paim on, nyprometose, tuterin, tycopene, maltodevirin managenese magnesium sulfate medium-chain triglucerides modified for starts
		narodexini, narganese, nagresami evitani chan orgeterese, noniner los anten, nacionamide, nickloss sulfate, phytonadione (vitanii K.), polyethytene dycol, polyvinyl alcohol
		potassium iodide, pregelatinized corn starch, pyridoxine hydrochloride (vitamin B6), riboflavin
		(vitamin B2), silicon dioxide, sodium ascorbate, sodium benzoate, sodium citrate, sodium
		metavanadate, sodium molybdate, sodium selenate, sorbic acid, stannous chloride, sucrose, thiamine
		mononitrate (vitamin B1), titanium dioxide, tricalcium phosphate, vitamin A acetate (vitamin A),
52	Folgers classic reast coffee	zme oxtde. May also contain less than 2% of: sodium aluminium silicate
54	Gerber Graduates Finger Foods cherry puffs	to by a pure conce
5.	oeroer onadaateoringer roodo enerry pano	concentrate, tri- and dicalcium phosphate, mixed tocopherols, natural flavor, zinc suffate, soy
		lecithin, alpha tocopheryl acetate (vitamin E), electrolytic iron
55	Ibuprofen	Ibuprofen USP, carnauba wax, cellulose, corn starch, FD&C Yellow No. 6, fumed silica gel,
		hypromellose, lactose, magnesium stearate, polydextrose, polyethylene glycol, sodium starch
56	One a day women's prepatal complete	gycolate, steante acid, titanium dioxide Calcium endporte a callulace, forecus fungente, magnacium exide, acacthic acid, disalcium phoephate.
50	prenatal multivitamin plus DHA	Cardini catomate, centios fundate, inducto anti accinative estatu prospitate,
	prenatur mantimum pros DTD P	polytinyl akohol, zinc vide, po-calcium pantothenate, corn starch, itanium dioxide, polyethylene
		glycol, magnesium stearate, talc, cupric sulfate, silicon dioxide, pyridoxine hydrochloride, calcium
		silicate, riboflavin, thiamine mononitrate, hypromellose, beta-carotene, FD&C Red No. 40 Lake,
		folic acid, vitamin A acetate, potassium iodide, cholecalciferol, cyanocobalamin. Liquid gel: marine
		lipid concentrate, gelatin, glycerin, methacrytic acid copolymer, propylene glycol, glyceryl
57	Halls Defense Supplement drops	monostearate, trietnyl otrate, triacein, soy technin Isomali sedium secortate, citica edito, astrona favorine, asecortic acid aspartame acesulfame
57	mans Defense Supprement utops	isonan, south accorder, circle acquire acquire acquire active act, apartance according to the south accorder (color). Red 40, sou lecitin
58	Women's vitamins	Not available
59	Clover honey	Honey
60	Spices	Salt, garlic salt, pepper, pickling spice, rosemary flakes, oregano, thyme flakes, dill weed, bay leaves, crushed red pepper, parsley flakes, paprika, cinnamon, all spice, ground mustard, celery salt, whole penper, minord onion
61	Triaminic Children's Thin Strips	perper, Interest of the sectore, alcohol, dibasic sodium phosphate, FD&C Red 40, flavors,
-		hydroxypropyl cellulose, hypromellose, maltodextrin, microcrystalline cellulose, polyacrilin,
		polyethylene glycol, pregelatinized starch, propylene glycol, purified water, sorbitol, sucralose,
		titanium dioxide
62	Mary Kay Timewise day solution	Not available
63	Mary Kay cleanser	Not available
65	Isopropyl rubbing alcohol	I YOU available 01% isomovi alcohol
66	Clorox daily sanitizing spray	Sodium hypohlorite 0.0095%, other ingredients 99.9905%
	,	

Number	Name	Ingredients, where available
67	Ron Bacardi rum	Not available
68	Golden Fields fiber	Out bran, wheat germ, psyllium seed husks, rice bran fiber, red wheat bran
69	Windshield washer fluid	Not available
70	Lemon Lime Gatorade	Sucrose syrup, glucose-fructose syrup, citric acid, natural lemon and lime flavors with other natural
		flavors, salt, sodium citrate, monopotassium phosphate, ester gum, Yellow 5
71	Rite-Aid aspirin	Aspirin, acetylated monoglycerides, anhydrous lactose, colloidal silicon dioxide, croscarmellose
		sodium, edible ink, FD&C Ked No. 40 Lake, FD&C Yellow No. 6 Lake, hypromeliose, hypromeliose
		primaraze, micros ystanine centrose, mineral or, polyeurytene grycor, polysoroate so, yenow non oxide
72	Chiavetta's marinade	Vinegar, salt, spices, fresh garlic, xanthan gum
73	Splenda no-calorie sweetener	Dextrose, maltodextrin, sucralose
74	Sugarfree Redbull	Carbonated water, sodium citrate, taurine, glucuronolactone, caffeine, acesulfame K, aspartame,
		inositol, xanthan gum, niacinamide, calcium pantothenate, pyridoxine HCl, vitamin B12, natural and
75	Monster Energy	artinicial navors, colors Contensited water gueres alwages gittin and antural flavors touring and an added
15	Wonster Energy	Caronaced water, sucrose, gracose, curie acu, natural navos, tatunne, sociari curiate, cono adueta,
		chloride, glucuronolactone, inositol, guarana seed extract, pyridoxine hydrochloride, sucralose,
		riboflavin, maltodextrin, cyanocobalamin
76	Tums cool relief	Calcium carbonate USP, Blue 1, corn starch, flavors, mineral oil, Red 40, sodium polyphosphate,
	N 1 1	sucrose, talc, Yellow 5 (tartrazine), Yellow 6
77	White sugar Negtle Negguite strengtherery	Sugar Sugar moledantein antifeiel Auvers site and Alexald Deck Dive 1 vitamins and minarals
79	Sesame Street Fizzy Tub Colors	sugar, manufactum, annuen navne, cum ann, even au, san, ener i vanamet annumentation solution annuen a
	Sesame Succer Party Tub Colors	benzoate, fragrance, FD&C Yellow 5, FD&C Blue 1, D&C Red 33.
80	L'Oreal Bare Naturale gentle mineral	Titanium dioxide, zinc oxide, talc, boron nitride, dimethicone, Copernicia cerifera (carnauba) wax, cetyl
1	makeup, classic tan 470	dimethicone, trimethylsiloxysilicate, triisocetyl citrate, Aloe barbadensis leaf juice, tocopherol,
	There F X 750 enterid/calsises see also at	panthenol. May contain: titanium dioxide, zinc oxide, bismuth oxychloride, iron oxide
81	Tums E-X 750 antacid/calcium supplement,	Calcium carbonate USP, sucrose, calcium carbonate, corn starch, fale, minerai oli, natural and artificial Bouera e divise esite codium redunderebate Bed 401 ede Volleur 51 ede Volleur 51 ede e
1	chewable tablets	Invols, autor actu, sourini polyphosphate, Red 46 Lake, Tenow 6 Lake, Tenow 5 (tartazine) Lake,
82	McCormick sprinkles	Not available
83	Tylenol Cold, multisymptom, daytime, rapid-	Acetaminophen, dextromethorphan HBr, phenylephrine HCl, benzyl alcohol, black iron oxide,
1	release gelcaps	butylparaben, carboxymethylcellulose sodium, colloidal silicon dioxide, corn starch, D&C Red
1		No. 28, D&C Yellow No. 10, edetate calcium disodium, FD&C Blue No. 1, FD&C Red No. 40,
1		getatin, hyprometiose, meniyiparaben, microcrystaliane celulose, polyenyiene giycoi, polysorbate
		sodium lauvi sultare sodium propionate sodium starch physical estaric acid. titanium dioxide.
79	Sesame Street Fizzy Tub	Colors Sodium bicarbonate, pentasodium triphosphate, succinic acid, citric acid, PEG 8, elycerin, denatonium
	besame buccer i hay rue	bound of the foregoing ED & C Valley 5, ED & C D & C D & 122
		benzoate, fragrance, FD&C fellow 5, FD&C Blue 1, D&C Red 55.
00	rounds underd, energy, desets	glycol, precelatinized starch. Red No. 27 Lake, sucrose
87	Raspberry coffee syrup	Cane sugar, glucose, raspberry juice concentrate, natural and artificial flavors, citric acid, less than 1/10
		of 1% sodium benzoate/potassium sorbate as a preservative
88	Pez, cherry	Sugar, corn syrup, adipic acid, hydrogenated palm kernel and palm oils and soybean oil mono- and
80	Mulicon infante' antigas drops	ugycenaes, natural and artificia inavors, artificia exist disparse malificial microcrystalline cellulose
09	wyncon mants anugas, urops	simetineone, carlossymethyleenuose sodum ( unte acto, navois, matter), met octystamie centrose,
90	Suave Naturals conditioner, tropical coconut	Cetyl alcohol, cetrimonium chloride, potassium chloride, fragrance, distearyldimonium chloride,
		disodium EDTA, glycerin, 2-bromo-2-nitropropane-1,3-diol, methylchloroisothiazolinone, methyl-
		isothiazolinone, propylene glycol, silk amino acids, honey (Mel), nettle (Urtica dioica) extract,
	F 11 11	rosemary (Rosmarinus officinalis) extract, coconut (Cocos nucifera) extract
91	French's mustard, honey	Distilled vinegar, high-fructose corn syrup, No. 1 grade mustard seed, honey, sait, modified food starch
92	Airborne hand lotion	(applea and conf), carlot extract (coor), spices, game powder
93	Softsoap antibacterial hand soap	Triclosan water, sodium laureth sulfate, cocamidopropyl betaine, decyl glucoside, fragrance, DMDM
		hydantoin, sodium chloride, PEG 120 methyl glucose dioleate, tetrasodium EDTA, sodium sulfate,
		polyquaternium-7, citric acid, poloxamer 124, PEG 7 gyceryl cocoate, D&C Red No. 33, FD&C Blue
		No.1
94	Nestle Good Start Supreme DHA and ARA	Corn maltodextrin, vegetable oil, enzymatic hydrolyzed soyprotein isolate, less than 1.5%: sucrose,
	soy man formula	Source of arachionic acid (ARA). C columit of ace, social extension cardia (DHA)].
		magnesium chloride, calcium chloride, protessium chloride, ferrous sulfate, copper
		sulfate, potassium iodide, sodium selenate, soy lecithin, monoglycerides, sodium ascorbate, choline
		chloride, inositol, alpha-tocopherol phosphate, niacinamide, calcium pantothenate, vitamin A
		(acetate), riboflavin, thianin mononitrate, pyridoxine hydrochloride, folic acid, biotin, phylloqui-
05	Linten oronoo horbol too	none, vitamin D3, vitamin B12, L-methionine, carrageenan, taurine, L-carniline
95	Lipton orange neroat tea	Contaige peet, nubseus nowers, ennianton, roasted enteory root, erove, neoree root, natural orange navor (contains sov lecitibin)
96	Mary Kay Timewise night solution	Not available
	· · · · · · · · · · · · · · · · · · ·	

# 4. Perform the experiment

### Perform the experiment

96 stock solutions were prepared by diluting the products with distilled deionized water.

Stock solutions were placed in a single 96 well, deep well plate, this plate was used as the mother plate.

Daughter plates were prepared by further diluting the mother plates with:

- 1. Water
- 2. Buffer (MES, HEPES, or Bis-Tris-Propane)
- 3. 25% PEG 3350

In total 8 unique 96 well source plates were prepared Original 100% Pseudo Silver Bullet (PSB)

1:10 Diluted PSB

- 100% PSB:25% PEG 3350 pH 5.81
- 100% PSB:25% PEG 3350 pH 6.81
- 100% PSB:25% PEG 3350 pH 7.79
- 1:10 D-PSB:25% PEG 3350 pH 5.81
- 1:10 D-PSB:25% PEG 3350 pH 6.81
- 1:10 D-PSB:25% PEG 3350 pH 7.79



### Crystallization Screening

The 20 proteins were set up against the eight different formulations of different solutions, two proteins in each 1536 well plate.

1	2	3	4
5	6	7	8
9	10	11	12
13	14	15	16
7 <mark>111 1111</mark>		50000 <b>1</b> 00000	abaalaha
Control of the local division of the local d		机的形构造成的复数分词运行	



### Crystallization Screening

The plates were imaged 1 day after setup then weekly for 6 weeks.

Results were scored for crystals





## 5. Analyze the data

	A	В		с		E	F	G	H		J	ĸ	$L \mid N$	I N	0	P	Q	R	S	Т	U	V	W.	X			Y			Z
1					12	22	12	23	12	4	12	5	126	12	27	12	8	12	29	13	30	1	31							
2					O	۷	A	н	J	L	Р	в	C D	Е	F	U	¥	м	N	R	Т	Q	S							
3	1536- Plate Well	Veep Vell Locatio	Pseudo Silve	r Bullet (PSB)	4 Wks	4 Wks	4 Wks	4 Wks	4 Wks	4 Wks	4 Wks	4 Wks	4 WKS 4 WKs	4 Wks	4 Wks	4 Wks	4 Wks	4 Wks	4 Wks	4 Wks	4 Wks	4 Wks	4 Wks		P	seudo	Sil <del>v</del> er B	ullet (PS	в)	Total Hits Per PSB
4	1	A1	Bul	bbles	5		7			2	5											7	1			27				
5	2	A2	Distilled W	'hite Vinegar	1		3			1	6											3				Distil	lled White	Vinegar		14
6	3	A3	Clorox Disi	nfect Cleaner	4	1	7		1	3	4	1						_				5				Cloro	x Disinfeq	st Cleaner		26
7	4	A4	Welch's Whit	te Grape Juice	1		5		1	3	2											5	1			Welch's	s White G	irape Juic	e	18
8	5	A5	Dover	Tairspray	3		4		1	3	Z	1										3	1			D	love Hair	spray		18
9	6	A6	Scope Mint	t Mouthwash	1		7		1	_	6	1		1		_						5		-		Scope	e Mint Me	outhwash		22
10	7	A7	Nyquil Liq	uid- Cherry	2		1		4	_	5			-		-		_				5	1		<u> </u>	Nyq	uil Liquid	Cherry		18
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### Proteinase K 4-Week Image

### Pseudo-Silver Bullet

### **Original Silver Bullet**



Fish Sauce 12.5% PEG 3350 HEPES pH 6.81 Silver Bullet H12 12.5% PEG 3350 HEPES pH 6.8

### **Concanavalin A 4-Week Image**

#### **Pseudo-Silver Bullet**

Distilled Vinegar, Water, High Fructose Corn Syrup, #1 Grade Mustard Seed, Sugar, Corn Syrup, Salt, Carrot Oleoresin (color), Honey, Spices, and Garlic Powder.



#### French's Honey Mustard 12.5% PEG 3350 HEPES pH 6.81

### **Original Silver Bullet**



Silver Bullet A8 12.5% PEG 3350 HEPES pH 6.8



Chemical diagrams for (a) tartrate used for crystallization of thaumatin and (b) ferrous fumarate found as an ingredient in prenatal vitamins, showing obvious chemical similarities.

#### The chemicals found in one Pseudo Silver **Bullet**

Aftershave lotion

The name, chemical structure and probable use of the non-PEG ingredients in PSB 41, aftershave lotion. Name Structure Use if known HO, \_OH Triethanolamine pH balancer Carbomer A product class consisting of high molecular weight polymers Thickening, dispersing, suspending and emulsifying agents

of acrylic acid

Antimicrobial preservative

Anti-fungal agent

Preservative

Moisturizer

Methylparaben

Diazolidinyl urea

Stearic acid

Propylparaben

Allantoin

BHT (butylhydroxytoluene)

Disodium EDTA (shown without the two sodium ions)

Coloring agent

Coloring agent

FD&C Yellow No. 5

Denatonium benzoate<sup>†</sup>

D&C Green No. 5

NaOO

ö NaO,S

Agent to denature ethanol

† Denatonium benzoate is listed somewhat cryptically on the ingredients as part of the ingredient SD alcohol 40, used to denature the ethanol.

Antioxidant additive

Possibly used to produce a pearlescent effect

Improves stability towards air, chelating agent







# 5. Supporting data

### **Differential Scanning Fluorimetry**

- We ran the PSB from the mother plate deep well through the Thermofluor with 20 different proteins to check for stabilization/ destabilization
- 20 proteins x 96 stock PSB's
- Twelve of the proteins run with the PSB gave distinct Tm values; some of the PSB stabilized the protein (the protein denatured at a higher temperature than the control)
- These twelve proteins were then run through Thermofluor with real SB to compare to the PSB



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4	1 38	D2	Lei	Lemon Juice							D			N	-		D	•		Ū	Ū	-					5	7	1	6			
4	2 39	D3	Ma	Maple Extract							•	-	-	-	-	-	-	-		-	•	-	-				1	0	0	18			
4	3 40	D4	Bals	Balsamic Vinegar							-	-	D	-	-	-	D	-		-	•	-	•	- C			1	3	0	15			
4	4 41	D5	Afta Aft	Afta Aftershave Lotion							D	U	N	D	N	-	U	DI	NC	U	Ν	D	D	) [	)		5	9	4	1			
4	5 42	D6	Heinz T	omato	o Keto	hup		U	-	U	D	U	D	D	D	-	U	UI	ο.	U	U	-	U	JC	)		9	6	0	4			
4	6 43	D7	Raspberr	ry Lem	-	U	D	-	D	υ	-	-	-	- 1	D -	-	D	-	-				3	6	0	10							

No.	Pseudo silver bullet (PSB)	С	G	E	H	K	0	Р	Q	R	S	T	No.	Pseudo silver bullet (PSB)	C	G	E	Н	K	0	Р	Q	R	S	Т	
1	Bubbles	-0.5	-0.1	-0.2		-1.0	-0.1	-2.6	-8.4	1	-0.7	-1.4	49	Cascade dish detergent	-4.3	-7.9	-21.9		-8.3		-4.5	2.0		-22.8		
2	Distilled white vinegar		-2.0	-0.1		-10.5		-20.4	4.7		-10.8		50	Equal sweetener	2.3	0.4	1.1		-0.2	-0.3	0.2	0.2	-0.6	0.8	1.5	
3	Clorox disinfect cleaner	-6.0	-11.2	-10.2			-2.3	-3.8	10.3			-9.5	51	Severe cold, daytime		-3.2	-1.6	-1.0	-10.3		-10.0	7.7	1	-16.0		
4	Welch's white grape juice	2.9	-0.9	5.5	0.5	-4.7		-0.2	1.2	10.4	-10.4	-12.4	52	Centrum multivitamin	2.6	1.1	2.6		-1.4	-1.5	28.2		-3.9	1,0	-1.2	
5	Dove hairspray							-1.3	2.8	-2.6			53	Folgers roast coffee		-2,5			-0.2	0.4	8.6	7.9	-1.4	6,3		
6	Scope mint mouthwash	1.6	0.0	0.1		-1.2	0,2	-0.3	14.1	-1.9	0.1	-1.8	54	Gerber cherry puffs	0.3	0.2	1		-0.1	0.5	0.7	-5.9	0.1	0.0	-0.7	
7	Nyquil Liquid- cherry					-0.4	0.6	5.2	-19.6	-1.0	2.7	1.7	55	Ibuprofen	50	0.1	-3.2		0.0	0.7	-0.2	40.	-9.0	0.0	0.6	
8	Proactive Revitalize toner				-0.6	-11.1		-21.2	0.1		10.6		56	Prenatal vitamins	1.6	0.3			-1.0	-0.4	23.7	5.2	10.4	5.2	-0.3	
9	Sudafed PE children	4.5	0.5	3.2		0.1	-2.3	3.6	2.4	-1.2	-0.3	1.1	57	Halls defense		510		11.9	-74		-3.0	21		11.0	$\leq$	
10	Red wine vinegar		15	0.8	-0.3	-10.5		-16.3	7.7		164		58	Women's vitamins	0.7	-0.2	33		-1.1	-1.9	30.2	1	4.6	1,4	2.1	
11	Coke Cola, diet	4.9	0.5	-3.3		0.5	-0.1	3.2	11.3	0.3	-3.3	-1.0	59	Wegman's honey	4.8	0.2	12		0.2	.0.4	25	56	0.1	2.9	2.0	5
12	Listerine fluoride rinse		-12.1	-2.5		-0.8	0.0	-1.0	24.8	-6.3	-2.1	-2.5	60	Spices	2.9	-2.2	1.6		0.2	0.1	4.5	-25.5	-0.5	0.9	8.5	
13	Dill spears juice	4.6	0.1	5.4	0.9	-4.4		-3.8	2.0	6.1	+7.0	1.3	61	Children's Thin Strips	-1.4	0.5	0.8		-1.0	0.5	0.5		-0.5	43	-1.8	
14	Diet peach grapefruit	4.6	0.1	4.0		0.0	-0.3	4.4	1.4	-1.1	0.5	0.6	62	MK Timewise day	-6.7	-5.2	-10.2	5.2	-4.8	-0.3	5.2	1	1.6	-5.0	-2.8	
15	Heinz cleaning vinegar	3.9	-2.3	-0.1	-1.0	-12.0		-22.2	2.5		-10.4	10000	63	MK Timewise 3-in-1			-7.7		0.2	0.2	0.9	1	-0.4	-1.6	-3.2	
16	Febreze fabric refresher	-5.0	-0.4	-7.1		0.2	1.0	5.8	9.7	-0.3	0.8	0.9	64	MK TW step 2: replenish	-3.2	-16.4	-7.6	5.0	0.0	-1.9	0.5	23	0.0	-0.1	1.1	
17	Contact solution	2.4	-0.1	0.1		-0.8	-0.2	0.2	-0.2	-0.2	-0.7	0.3	65	Rubbing alcohol	-0.4	-1.7	-0.3		-3.3	-0.5	-1.4	4.4	-1.4	-0.7	-0.9	
18	Hydrogen peroxide	0,2	0.0	0.3		-0.8	-1.7	0.8	-33.2	-2.9	-0.6	-0.5	66	Clorox sanitizing spray	-2.0	-0.1	0.2		0.2	-0.2	0.3		-0.2	0.2	-0.1	
19	Tops fat-free milk	-3.2	1.5	5.5		-0.2	0.2	2.2	2.9	0.2	-3.3	-0.2	67	Ron Bacardi rum	-0.3	-0.3	-0.2		-1.2	-0.4	-0.4	-	-0.6	-0.2	0.2	
20	Deep-cleaning astringent		1			-1.2	-5.7	4.0	5.9	-3.3	-5.1		68	Golden Fields fiber	2.2	-2.8	-0.7		-0.1	-0.1	2.5	-42	0.2	0.1	0.0	
21	Margarita mix	0.9	-11.7	-13.4		-12.2		+11.8	1.9		-15.6	-11.1	69	Windshield washer fluid	0.0	0.0	-0.1		-0.6	0.2	-0.6		0.8	0,3	-1.2	
22	Tiparos fish sauce	3.5	1.9	1.5		0.2	-1.0	5.5	-2.1	-0.4	-1.1	-1.2	70	Lemon Lime Gatorade		-1.3	3.6		-5.2		1.8		1,5	-9.7	1.7	
23	Body spray, vanilla		1	12 1		0.3	0.9	-0.4	-3.2	-2.9			71	Rite-Aid aspirin	-4.2	-2.0	1.9		-4,4		-3.5		5.8	-11.1	2.7	
24	Bathroom cleaner				-1.5	-14.4		-22.7	2.6		-9.1		72	Chiavetta marinade BBQ	-1.5	-4.1	-3.8	-0.7	-12.2		+16.7	7.0		-21.1		
25	Polish remover, regular	0.3	-0.1	0.3		-0.8	0.2	-0.5	-1.0	-0.2	0.3	-0.2	73	Splenda sweetener	0.5	0.3	0.1		0.3	0.0	2.2	-3.6	-0.8	0.2	-0.4	
26	Red food coloring	2.8	The						-0.7				74	Sugarfree Redbull		-0.9		0.3	-4.0		3.5	3.5	6.6	-6.4	3.0	
27	2-in-1 body shampoo		-				<u> </u>		-0.9			-	75	Monster Energy		-0.3	12		-1.4		5.0	11.3	1.2	-6.0	0.7	
28	Gel dishwash detergent	55	1 25			10 O		0.00	1.0	1.1		6 8	76	Tums cool relief	-0.7	0.6	-0.3		-0.1	1.1	3,3	-63	-0.1	-1.5	-1.7	
29	Purell hand sanitizer	-7.9	-0.1	.9.3		0.0	0.4	2.0	2.1	-25	-0.2	-6.1	77	Sugar, white, granulated	0.4	0.2	0.1		0.0	0.2	1.2	-43	-0.1	0.2	0.1	
30	Airborne baby wash				-				-0.4	Alte			78	Nesquik strawberry	0.0	0.4	0.1		-0.1	0.6	4.5	10.7	0.7	3.0	0,4	
31	Dawn dishwash liquid		-			-		-	-12	-		-	79	Fizzy Tub Colors	11.7	-2.4	-8.0		-0.4	-2,4	11.2	-5.0	-3.1	-9,3	-8.7	
32	Excedrin	-8.6	.27	0.3	0.0	-8.5		-14.1	-2.3		-17.7	1.9	80	L'Oreal mineral blush	0.5	0.2	-0.5		0,1	1.4	1.6	-0.8	0,4	-0.8	-2.5	
33	Cinnamon mint	1.6	0.4	0.3		0.1	0.7	13	4.2	-3.1	0.9	-0.8	81	Tums antacid	-1.5	1.1	-0.3		-0.4	0.9	2.6	4.4	0.3	-0.6	-3.4	
34	Weamans dish deteraent								4.0			010	82	Cake Mate sprinkles	1.2	0.2	0.9		0.2	0.8	0.6	48	0.1	1.0	-0.1	
35	Baking soda	2.2	-5.0	-13.9		-27	-16.6	3.7	11.2	-85	.142	-15.8	83	Tylenol cold, daytime	0.2	0.2	0.4		-0.9	0.5	0.2	-55	-13	-1.2	-0.5	
36	Cranherry nomeoranate			1010			1010		3.0	- Mile	A THE	1010	84	Omega 3 fish oil	-0.6	-0.2	0.2		0.0	0.0	0.0	0.0	-0.1	-0.6	-0.9	
37	Tilex mildew remover		.9.4	.13.6		1	.5.9		8.7		.153	.143	85	Cold-Eeze lozenges		0.3	5.2	0.6	-6.0	-16.2	-1.5	-12				
38	Lemon juice	-	42	-7.2	0.2	-14.4		-20.0	13.1	-	-255	-9.0	86	Rolaids antacid, cherry	-0,4	1.2	0.1		-0.4	0.1	3.2	4.1	-0.2	-1.7	-4.6	
39	Manle extract		tuis	7.10	8.10				15.0		avie	7.0	87	Raspberry coffee syrup		-0.2	4.9		-0.3	-2.6	6.1	4.6	-1.9	4.6	1.9	
40	Ralcomic vineoar		.22			-	-	.12.5	41		-14.6	1	88	Pez, cherry	3.2	0.3	0.4		0.0	-0.1	1.1	-[1.2	-0.2	0.0	0.0	
41	Afta aftershave lotion		.25	-27.4	-	0 T	-68	- 1.0-14 <sup>*</sup>	12.2	1115	-20.8	0.0	89	Infants' antigas, drops	1.8	-7.7	-23	-155	-0.2	0.9	3.4	4.7	-0.2	0.1	-0.6	
47	Heinz tomata ketchun	2.0	44	25	0.4	-4.4	-20.9	.0.2	-0.5	41	10	48	90	Coconut conditioner	+11.7	-0.3	-1.8	6.7		-0.1	-3.8	0.2		-8.6	-3.3	
43	Rashberry lemonade miy	4117		110	6.0	156	SAVIA.	-V-8	67	1.1	1.0	1.0	91	French's mustard, honey	-1.0	-4,4	3.7	0.1	4.2		5.1	4.9	3.4	-6.9	3.3	
44	Ground black nennes	2.8	0.7	-14		-0.2	11	27	121	-0.1	12	5.8	92	Airborne hand lotion	4.6	-0.3	0.1		0.0	0.2	0.4		-0.5	0.3	0.0	
45	Powder dish deteraent	410	017	20.7		7.0	147	33	11.6	-364	1.00	2.0		Control point		0.0	0.0		0.0	0.0		0.0	0.0	0.0	0.0	
46	Ground cinnamon	28	-0.2	17		0.4	13	2.0	-1.0	153	63	0.0	0.4	Good Start surrame	20	0.0	1.8		.03	0.1	3.7	164	0.4	37	.0.4	
47	Weamans salt indized	11	0.0	0.8		0.1	0.5	45	27	121	11	-31	94	Linton arange tes	.00	3.5	10		-0.5	12	25	55	0.7	63	0.9	
49	Avon loose nourder	0.0	0.0	0.1	-	6.1	0.0	0.9	and .	14.4	.0.4	.0.0	95	MK Timewise night	55	-0.5	0.0	5.0	0.1	.05	1.0	0.9	4.2	40.3	0.0	
40	reton toose porrete	A16	1/104	304		Wal	010	0.0		-0.0	-0.4	-0.7	90	sure chinewise inglit	"dial	-1.1	23.00	- 20	Week	"Und	0.0	Vs7	1.1.44		-0.0	

### Communicate Results



Each of the students presents to an audience of scientist and peers.

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Received 15 June 2010 Accepted 12 July 2010 Crystal cookery – using high-throughput technologies and the grocery store as a teaching tool

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Crystallography is a multidisciplinary field that links divergent areas of mathematics, science and engineering to provide knowledge of life on an atomic scale. Crystal growth, a key component of the field, is an ideal vehicle for education. Crystallization has been used with a 'grocery store chemistry' approach and linked to high-throughput remote-access screening technologies. This approach provides an educational opportunity that can effectively teach the scientific method, readily accommodate different levels of educational experience, and reach any student with access to a grocery store, a post office and the internet. This paper describes he formation of the program through the students who helped develop and p ototype the procedures. A summary is presented of the analysis and preliminary results and a description given of how the program could be linked with other aspects of crystallography. This approach has the potential to bridge the gap between students in remote locations and with limited funding, and access to scientific resources, providing students with an international-level research experience.

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Also - Goes on resume for college application!

Where appropriate they help write up the results and receive authorship for their work. This has one of the biggest impacts in encouragement and motivation.

#### THE SCIENTIFIC METHOD ... FOR TEN-YEAR OLDS



\* THIS IS SUPPRISINGLY CLOSE TO HOW REAL SCIENTISTS ACT AT CONFERENCES.

# 6. The future

### In progress – this years students

Do grocery store items mimic silver bullets?

Crystallize a subset of proteins with silver bullets and identified pseudo silver bullets.

Collect diffraction data.

Process the data.

Refine the structure and determine the ligand if any.

### Where we would like to take it ....

Crystallization screening is virtual – results are seen over the internet

Identify a school or schools that can simply do the experiment of selecting a number of different compounds

Run the screening over the internet with the school simply sending a 96 well plate filled with solutions.

Monitor results.

Send one of our scientist to the school to go over the analysis of results.

In the process teach the scientific method and get children to discover discovery.

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