

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

Joseph R. Luft,^{a,b} Nicholas M. Furlani,^{a,c,‡} Rachel E. NeMoyer,^{a,d,‡} Elliott J. Penna,^{a,e,‡} Jennifer R. Wolfley,^a M. Elizabeth Snell,^a Stephen A. Potter^a and Edward H. Snell^{a,b,*}

^aHauptman–Woodward Medical Research Institute, 700 Ellicott Street, Buffalo, NY 14203, USA, ^bDepartment of Structural and Computational Biology, SUNY Buffalo, 700 Ellicott Street, Buffalo, NY 14203, USA, ^cCase Western Reserve University, Cleveland, OH 44106, USA, ^dPennsylvania State University, University Park, PA 16802, USA, and ^eLehigh University, Bethlehem, PA 18015, USA. Correspondence e-mail: esnell@hwi.buffalo.edu

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 the formation of the program through the students who helped develop and prototype 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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1. Introduction

Crystallography is a multi-disciplinary field that links divergent areas of mathematics, science and engineering to provide knowledge of life on an atomic scale. The results are important from both scientific and biomedical aspects, with some 24 Nobel prizes in the field. From an educational point of view, crystals are visually stunning and their atomic structures readily convey the concept of functional attributes being dictated by structural features. Students are familiar with biological processes such as the Krebs cycle. The crystallographic structures of the enzymes involved in such cycles provide students with a more comprehensive and deeper understanding of these complex biological processes. Memorizing the names of enzymes and substrates involved in a pathway does not convey the same level of understanding as seeing the structures of the molecular machines that make them possible. At the Hauptman–Woodward Medical Research Institute (HWI), where one of our core focuses is crystallography, we have a summer internship program that offers undergraduate students an opportunity to spend ten weeks on a scientific project, participating in ongoing research at the laboratory. The students interact directly with scientists,

graduate students, laboratory personnel and other students in a laboratory environment to experience fully the scientific process from a variety of perspectives. During the past four years, approximately 80 students from over 20 different colleges, polytechnics and universities have taken part in HWI student internships. As part of this program we have taken a key step in the science of crystallography, namely crystallization, and used this step to develop an educational study that centers on the chemistry of crystallization but more importantly teaches students the scientific method.

In the scientific method we begin with a question. Background research is conducted to build a hypothesis surrounding this question, and then the hypothesis is tested. The data are analyzed, the hypothesis reviewed and eventually the result is communicated. We have developed a program where we enable students to experience the scientific method by introducing an initial hypothesis and letting them design the experiments to test the hypothesis, perform those experiments, analyze the results and then refine the hypothesis. We enable them to participate directly in the ownership of the discovery process. All too often students are exposed to an illusion of science, being presented with a collection of preconceived facts to be memorized (not the scientific process) (Linn & Songer, 1993; Richmond & Kurth, 1998).

‡ A summer intern at HWI.

They learn these facts and solve problems that often have no real connection to the data, as they are created by educators and textbooks to teach the facts. The students hear about scientific practice but have limited active participation (Barab & Hay, 2001). We have developed a program that overcomes this by challenging the students to participate actively and undertake a biochemical investigation using the scientific method.

Macromolecular crystallization is a process whereby a solution of biological macromolecules is taken to a high degree of supersaturation. The molecules undergo an ordered aggregation followed by a period of sustained growth to yield a crystal. Many experimental approaches have been developed to drive the macromolecule to a state of supersaturation (Luft & DeTitta, 2009). Typically, a macromolecule, in an appropriate buffer, gradually undergoes chemical or physical variation of the solution environment, reducing the solubility of the macromolecule in order to drive the system to a state of labile supersaturation, both thermodynamically and kinetically favorable to promoting nucleation. While supersaturation of crystallization experiments is most commonly achieved using chemical precipitants, variables such as pH (McPherson, 1995), temperature (Astier & Veesler, 2008) and even pressure (Visuri *et al.*, 1990) have been used to promote crystallization. An alternative crystallization strategy, Silver Bullets (Hampton Research, Aliso Viejo, California, USA), uses libraries of small molecules (inorganic and organic salts, organic acids, biological ligands, amino acids, and peptides) to stabilize and promote lattice contacts in order to crystallize biological macromolecules using a very limited set of supersaturating conditions (Larson *et al.*, 2007a; McPherson & Cudney, 2006; McPherson *et al.*, 2007). The macromolecules select specific chemically relevant small molecules from these multiple component solutions. The Silver Bullets approach significantly increases the number of crystallization hits compared with control solutions where the small molecules were absent. We have built on these results to design an educational program that makes use of readily available effective and non-hazardous formulations of small-molecule libraries (grocery store products) that can be chosen by students. We combine the student-selected libraries of small molecules with high-throughput crystallization screening technologies, data acquisition and analysis.

Many items found in grocery store products (or in the home) are established and commonly used crystallization precipitants [*e.g.* salts, polyethylene glycols (PEGs)], while others contain components identified in promoting crystallization, *e.g.* in an extreme case, Sweet & Snappy Classic brand pickle juice being used successfully to crystallize a mutant protein (Hickey, 1999). Pickle juice contains glycerol, PEG 400, citric acid, acetic acid, alum and vitamins. These ingredients have all been used to promote crystallization and the components have been observed in crystal structures bound to biological macromolecules. The questions we set for our students were how do these small molecules affect proteins and can they be used as successful agents for crystallization? With these broad questions we also set limits, selecting a group

of 20 commercially available proteins with a range of biochemical and biophysical properties as our test set, and instructed the students to select ~100 different liquid or soluble products that could be bought off the shelf in a grocery store (or were readily available at home).

We provided the initial question and limited boundary conditions. The students built a hypothesis around the question. They tested this hypothesis, analyzed their results, reviewed the hypothesis and eventually communicated the outcome. In this paper we describe the process, the results and how we envisage the future development of this strategy as an educational tool. We view the program as an opportunity to build students' enthusiasm for science through active participation and exposure to research that teaches the scientific method, and to open students' eyes to the world of collaborative science on a national and international level.

2. Experimental

2.1. Protein targets

The 20 protein targets are listed in Table 1. These proteins were chosen to cover a broad range of biochemical and biophysical properties, with molecular weights ranging from 9 kDa (ubiquitin) to 480 kDa (urease) and a correspondingly broad coverage of isoelectric points ($4.4 \leq \text{pI} \leq 10.7$). All of these proteins were available commercially at reasonable cost. Proteins were prepared in 50 mM NaCl and 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer at pH 7.4, except for proteins O and Q, which were prepared at pH 6.8, protein S, which was prepared in H₂O alone (it is shipped in this state) and protein G, which was in 20 mM citric acid at pH 3.0. Protein O has a pI of 7.4, making it relatively insoluble in pH 7.4 buffer, protein Q aggregates above pH 7.0, protein M is shipped dissolved in H₂O and protein G is stable at pH 3.0, hence the respective choices of solution conditions.

2.2. Small-molecule components

The small-molecule additives, termed pseudo-silver bullets (PSBs), were selected by the students from common products available in the grocery store. These products are pre-formulated, often in solution, and provide ready access to a diverse chemical library at low cost. They are sold without restrictions in the grocery store; they are relatively safe for students to use in the laboratory. Over 100 products were initially chosen and from these 96 were used in the final study. The final selection was based on the students' ability to prepare solutions of the products for the experiments described below. The primary source of ingredient data was the product packaging. In the USA where the experiments were conducted, the Food and Drug Administration (FDA) enforces the regulations for product labeling. In balancing the need for an informed consumer and manufacturers' trade secrets, the FDA requires that all ingredients be declared but not the proportion or percentage of each in the product. To illuminate the situation a little further the ingredients are

listed in descending order of their predominance according to weight. There are exceptions to these regulations: medicine labels list ingredients in alphabetical order of active and inactive ingredients, and collective language can be used for flavors and spices. Similarly, incidental additions can also be present and do not appear on the label. These are not considered ingredients if they are present at 'insignificant levels' and have no 'technical or functional effect' in food. There is often more in a product than the label might imply. Besides the product container there are additional sources of information. The US National Institutes of Health (NIH), through the National Library of Medicine (NLM), maintains a household products database (currently at <http://householdproducts.nlm.nih.gov>) that links information on the product label to its Chemical Abstract Service registry number and to literature on biological interactions. Information on food additives and pharmaceuticals is available elsewhere, e.g. the Code of Federal Regulations title 21, parts 170–199 (Wexler, 2004), and from the NLM at the drug information database (currently at <http://www.nlm.nih.gov/medlineplus/druginformation.html>). The product information and these databases were used by the students to construct Table 2, a listing of the chemical components in the 96 PSBs, the first step in our educational process. The students used online resources, developed careful note-taking skills and organized the data in a spreadsheet, and during this process became informed consumers.

The selection of products by the students fully engaged their participation in, ownership of and enthusiasm for the project. The products came in several forms: liquids, solids, powdered solids and combinations, e.g. a solid pill containing a liquid or powder. The students were challenged to develop robust generally applicable protocols to prepare reproducibly 5 ml of concentrated stock solutions for each of these compositionally diverse products. For liquid products, 5 ml of the product was dispensed into 5 ml of double-distilled (dd) H₂O and a vortex mixer used to make the solution homogeneous. The liquid was centrifuged (10 min at 8000 r min⁻¹), and 5 ml of the supernatant was removed and stored in a glass vial. In a minority of cases, sediment was observed after centrifugation, which was noted and discarded. This procedure gave the stock 100% PSB solutions used for the experiments. Solid products were crushed using a mortar and pestle. The resultant powder was transferred to a centrifuge tube, 10 ml of dd H₂O added to the tube, and a homogeneous stock solution prepared as described for the liquid products. In the case of

Table 1

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

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

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

powdered products, 1 g of the sample was diluted with dd H₂O to a total volume of 10 ml and a homogeneous stock solution prepared as described for the liquid samples. The remaining products were liquid-filled capsules. The capsules were pierced and the liquid extracted until 2 ml had been collected. The capsule liquid was dispensed into 2 ml of dd H₂O and a homogeneous stock solution prepared as described for the liquid samples. All stocks were initially stored at room temperature.

Small aliquots (100 μ l) of the stock solutions were transferred from room temperature to 277 K to determine the viability of long-term storage at lower temperature. The majority of the solutions did not form a visible precipitate. Those that did that form a precipitate after overnight storage at 277 K were filtered with a 0.45 μ m syringe filter. If precipitate was still observed, then this was followed by 0.2 μ m filtration.

Source (mother) plates of the product stock solutions were prepared (Fig. 1). A total of 500 μ l of each stock solution was dispensed into a 96-deep-well source plate. The source plate was sealed and stored at 277 K. No attempt was made to evaluate the final concentrations of the grocery store products in the stock solutions or to characterize them in any other way.

2.3. Differential scanning fluorimetry

Differential scanning fluorimetry (DSF) was used to measure the melting temperatures (T_m) of the 20 proteins in the presence of the 96 product-based additives. This method has been used to identify ligands that promote thermal

teaching and education

Table 2

Grocery store products and their ingredients.

National brands are listed with their brand names. Store-specific brands are listed with generic names. Water has not been listed in the ingredients unless it is carbonated or treated in some manner. In some cases the contents of the product were not available; these products were still included as a teaching tool for the design of an effective experiment.

| Number | Name | Ingredients, where available |
|--------|---|--|
| 1 | Bubbles | 0.5 cup dish soap, 1.5 cups water, 2 teaspoons sugar |
| 2 | White vinegar | Select sun-ripened grain, diluted with water |
| 3 | Clorox kitchen cleaner | Not available |
| 4 | White grape juice | White grape juice, citric acid, ascorbic acid, potassium metabisulfite |
| 5 | Dove Weightless Moisturizers hairspray with natural movement – extra hold | 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 officinalis</i> seed oil (<i>Borago officinalis</i>), palmitic acid, stearic acid, linoleic acid, oleic acid, eicosenoic acid, glycerin |
| 6 | Scope mouthwash | Alcohol, glycerin, flavor, polysorbate 80, sodium saccharin, sodium benzoate, cetylpyridinium chloride, benzoic acid, Blue 1, Yellow 5 |
| 7 | Nyquil liquid, cherry flavored | 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, sodium citrate |
| 8 | Proactive Solution revitalizing toner | Glycolic acid, <i>Hamamelis virginiana</i> (witch hazel) extract, <i>Anthemis nobilis</i> flower extract, <i>Rosa canina</i> fruit extract, <i>Aloe barbadensis</i> 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), Yellow 5 (CI 19140) |
| 9 | Sudafed PE children's nasal decongestant | Phenylephrine HCl, citric acid, edetate disodium, FD&C Red No. 40, flavor, glycerin, sodium benzoate, sodium carboxymethylcellulose, sodium citrate, sorbitol, sucralose |
| 10 | Red wine vinegar | Red wine vinegar (reduced with water to 5% acidity), potassium metabisulfite |
| 11 | Coca Cola, Diet | 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, FD&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 | Febreze fabric refresher | Alcohol, odor eliminator derived from corn, fragrance |
| 17 | Alcon Opti-Free disinfecting solution | Sodium chloride, sodium borate, propylene glycol, Tearglyde proprietary dual-action reconditioning system (Tetric 1304, nonanoyl ethylenediaminetriacetic acid) with Polyquad (polyquaternium-1) 0.001% and Aldox (myristamidopropyl dimethylamine) |
| 18 | Hydrogen peroxide | Stabilized hydrogen peroxide 3% |
| 19 | Fat-free milk | Fat-free milk, vitamin A, vitamin D3 |
| 20 | Clean & Clear deep-cleaning astringent | Salicylic acid, alcohol (24.5%), algae extract, <i>Aloe barbadensis</i> leaf extract, benzophenone-4, denatonium benzoate, dimethicone propyl PG-betaine, fragrance, glycerin, isoceteth-20, PEG 32, propylene glycol, sodium citrate, Blue 1 |
| 21 | Margaritaville margarita mix | High-fructose corn syrup, citric acid, sodium citrate, sodium benzoate, cellulose gum, gum acacia, polysorbate 60, natural flavors, glycerol ester of wood rosin, sodium metabisulfate, Yellow 5, Blue 1. |
| 22 | Tiparos fish sauce | Anchovy 60%, salt 37%, sugar 3% |
| 23 | Avon Naturals body spray | Fragrance(s)/perfume(s), ethanol/SD alcohol 40 |
| 24 | Antibacterial Scrubbing Bubbles II | Tetrasodium EDTA, isobutane, diethylene glycol monobutyl ether, dialkyldimethylammonium methyl sulfate |
| 25 | Pretty Nails polish remover | Acetone, lanolin, vitamin E, gelatin, fragrance, panthenol, glycerin, diglycerol |
| 26 | McCormick red food color | Propylene glycol, FD&C Red 40 and 3, 0.1% propylparaben |
| 27 | L'Oreal Kids 2-in-1 body shampoo for delicate skin, burst of green pear | Sodium lauryl sulfate, PEG 200 hydrogenated glyceryl palmate, disodium cocoamphodiacetate, PEG 30 glyceryl cocoate, glycerin, polysorbate 20, sodium chloride, PEG 7 glycerin cocoate, hexylene glycol, FD&C Yellow No. 5, FD&C Blue No. 1, fragrance, pear (<i>Pyrus communis</i>) extract, sodium lauryl-8 sulfate, sodium oleth sulfate, sodium methylparaben, DMDM hydantoin, magnesium lauryl-8, magnesium oleth sulfate, polyquaternium-10, propylene glycol, disodium EDTA |
| 28 | Palmolive gel dishwasher detergent | Phosphorus content: averages 1.6% phosphorous, in the form of phosphate, sodium hydroxide, sodium hypochlorite |
| 29 | Purell instant hand sanitizer | Isopropyl alcohol, glycerin, carbomer, fragrance, aminomethyl propanol, propylene glycol, isopropyl myristate, tocopheryl acetate |
| 30 | Airborne baby wash | Not available |
| 31 | Dawn Plus dishwashing liquid | Biodegradable surfactants (anionic and nonionic), enzymes |
| 32 | Excedrin | Acetaminophen, aspirin, caffeine, benzoic acid, carnauba wax, hydroxypropyl cellulose, hydroxypropyl methylcellulose, microcrystalline cellulose, mineral oil, polysorbate 20, povidone, propylene glycol, simethicone emulsion, sorbitan monolaurate, stearic acid, FD&C Blue 1, titanium dioxide |
| 33 | Brach's Star Brites cinnamon mint candy | Corn syrup, sugar, artificial and natural flavor, titanium dioxide color, Red 40, Red 3, Blue 1, Yellow 6 |
| 34 | Dish detergent, lemon-scented | An aqueous solution of anionic and nonionic surfactants, stabilizers, perfume, colorant and quality control agents |
| 35 | Arm & Hammer pure baking soda | Sodium bicarbonate |

Table 2 (continued)

| 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 | Balsamic vinegar | Balsamic vinegar |
| 41 | Afta aftershave lotion | SD alcohol 40, PEG 8 stearate, PPG-2 myristyl ether propionate, fragrance, triethanolamine, carbomer, diazolidinyl urea, methylparaben, PEG 2 stearate SE, stearic acid, propylparaben, allantoin, BHT, disodium EDTA, 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 powder, natural flavors |
| 43 | Crystal Light raspberry lemonade mix | Citric acid, potassium citrate, calcium phosphate, aspartame (phenylketonurics: contains phenyl-alanine) (sweetener), raspberry juice solids, natural and artificial flavor, acesulfame potassium (sweetener), soy lecithin, artificial color, Red 40, Blue 1, tocopherol |
| 44 | Ground pepper | Black pepper |
| 45 | Powder dishwasher detergent | Sodium carbonate, sodium silicate, enzymes |
| 46 | Ground cinnamon | Ground cinnamon |
| 47 | Salt, iodized | Salt, calcium silicate, dextrose, potassium iodide |
| 48 | Avon Personal Match loose powder | Not available |
| 49 | Cascade automatic dishwasher detergent | Fragrance(s)/perfume(s), surfactants, sodium silicate, sodium sulfate anhydrous, cleaning agents, colorant/pigment/dye, bleach, sudsing agent, water softeners (complex sodium phosphates/sodium carbonate) |
| 50 | Equal sweetener | Dextrose with maltodextrin, aspartame |
| 51 | Theraflu severe cold, daytime, natural lemon | Acetaminophen, phenylephrine HCl, acesulfame K, citric acid, D&C Yellow 10, FD&C Yellow 6, lecithin, maltodextrin, natural flavors, silicon dioxide, sodium citrate, sucrose, tribasic calcium phosphate |
| 52 | Centrum multivitamin/multimineral supplement, tablets | Dibasic calcium phosphate, magnesium oxide, potassium chloride, calcium carbonate, microcrystalline cellulose, ascorbic acid (vitamin C), ferrous fumarate, DL-alpha 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), DL-alpha tocopherol, FD&C Yellow 6 Aluminium Lake, folic acid, gelatin, hydrogenated palm oil, hypromellose, lutein, lycopene, maltodextrin, manganese, magnesium sulfate, medium-chain triglycerides, modified food starch, niacinamide, nickelous sulfate, phytonadione (vitamin K), polyethylene glycol, 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), zinc oxide. May also contain less than 2% of: sodium aluminium silicate |
| 53 | Folgers classic roast coffee | 100% pure coffee |
| 54 | Gerber Graduates Finger Foods cherry puffs | Rice flour, whole-grain oat flour, wheat starch, wheat flour, sugar, cherry powder, cherry juice concentrate, tri- and dicalcium phosphate, mixed tocopherols, natural flavor, zinc sulfate, 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 glycolate, stearic acid, titanium dioxide |
| 56 | One a day women's prenatal complete prenatal multivitamin plus DHA | Calcium carbonate, cellulose, ferrous fumarate, magnesium oxide, ascorbic acid, dicalcium phosphate, DL-alpha tocopheryl acetate, croscarmellose sodium, maltodextrin, niacinamide, gelatin, acacia, polyvinyl alcohol, zinc oxide, D-calcium pantothenate, corn starch, titanium 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, methacrylic acid copolymer, propylene glycol, glyceryl monostearate, triethyl citrate, triacetin, soy lecithin |
| 57 | Halls Defense Supplement drops | Isomalt, sodium ascorbate, citric acid, natural flavoring, ascorbic acid, aspartame, acesulfame potassium, beta carotene (color), Red 40, soy lecithin |
| 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 pepper, minced onion |
| 61 | Triaminic Children's Thin Strips | Dextromethorphan HBr, acetone, 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 |
| 64 | Mary Kay microdermabrasion | Not available |
| 65 | Isopropyl rubbing alcohol | 91% isopropyl alcohol |
| 66 | Clorox daily sanitizing spray | Sodium hypochlorite 0.0095%, other ingredients 99.9905% |

Table 2 (continued)

| Number | Name | Ingredients, where available |
|--------|--|---|
| 67 | Ron Bacardi rum | Not available |
| 68 | Golden Fields fiber | Oat 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 Red No. 40 Lake, FD&C Yellow No. 6 Lake, hypromellose, hypromellose phthalate, microcrystalline cellulose, mineral oil, polyethylene glycol, polysorbate 80, yellow iron 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 artificial flavors, colors |
| 75 | Monster Energy | Carbonated water, sucrose, glucose, citric acid, natural flavors, taurine, sodium citrate, color added, <i>Panax ginseng</i> root extract, L-carnitine, caffeine, sorbic acid, benzoic acid, niacinamide, sodium 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, sucrose, talc, Yellow 5 (tartrazine), Yellow 6 |
| 77 | White sugar | Sugar |
| 78 | Nestle Nesquik strawberry | Sugar, maltodextrin, artificial flavors, citric acid, Red 40, salt, Blue 1, vitamins and minerals |
| 79 | Sesame Street Fizzy Tub Colors | Sodium bicarbonate, pentasodium triphosphate, succinic acid, citric acid, PEG 8, glycerin, denatonium benzoate, fragrance, FD&C Yellow 5, FD&C Blue 1, D&C Red 33. |
| 80 | L'Oreal Bare Naturale gentle mineral makeup, classic tan 470 | Titanium dioxide, zinc oxide, talc, boron nitride, dimethicone, <i>Copernicia cerifera</i> (carnauba) wax, cetyl dimethicone, trimethylsiloxysilicate, triisocetyl citrate, <i>Aloe barbadensis</i> leaf juice, tocopherol, panthenol. May contain: titanium dioxide, zinc oxide, bismuth oxychloride, iron oxide |
| 81 | Tums E-X 750 antacid/calcium supplement, chewable tablets | Calcium carbonate USP, sucrose, calcium carbonate, corn starch, talc, mineral oil, natural and artificial flavors, adipic acid, sodium polyphosphate, Red 40 Lake, Yellow 6 Lake, Yellow 5 (tartrazine) Lake, Blue 1 Lake |
| 82 | McCormick sprinkles | Not available |
| 83 | Tylenol Cold, multisymptom, daytime, rapid-release gelpacs | Acetaminophen, dextromethorphan HBr, phenylephrine HCl, benzyl alcohol, black iron oxide, butylparaben, carboxymethylcellulose sodium, colloidal silicon dioxide, corn starch, D&C Red No. 28, D&C Yellow No. 10, edetate calcium disodium, FD&C Blue No. 1, FD&C Red No. 40, gelatin, hypromellose, methylparaben, microcrystalline cellulose, polyethylene glycol, polysorbate 80, powdered cellulose, pregelatinized starch, propylene glycol, propylparaben, red iron oxide, sodium lauryl sulfate, sodium propionate, sodium starch glycolate, stearic acid, titanium dioxide, yellow iron oxide |
| 84 | Spring Valley omega 3 fish oil | Gelatin, glycerin, anchovy, sardine, mackerel, fish oil concentrate, D-alpha tocopherol, D-sorbitol |
| 85 | Cold-Eeze cold remedy lozenges | Zincum gluconicum, corn syrup, glycine, natural flavors, sucrose |
| 86 | Rolaids antacid, cherry, tablets | Calcium carbonate, magnesium hydroxide, dextrose, flavoring, magnesium stearate, polyethylene glycol, pregelatinized 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 diglycerides, natural and artificial flavors, artificial colors FD&C Red 3 |
| 89 | Mylicon infants' antigas, drops | Simethicone, carboxymethylcellulose sodium, citric acid, flavors, maltitol, microcrystalline cellulose, purified water, sodium benzoate, sodium citrate, xanthan gum |
| 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, methylisothiazolinone, propylene glycol, silk amino acids, honey (Mel), nettle (<i>Urtica dioica</i>) extract, rosemary (<i>Rosmarinus officinalis</i>) extract, coconut (<i>Cocos nucifera</i>) extract |
| 91 | French's mustard, honey | Distilled vinegar, high-fructose corn syrup, No. 1 grade mustard seed, honey, salt, modified food starch (tapioca and corn), carrot extract (color), spices, garlic powder |
| 92 | Airborne hand lotion | Not available |
| 93 | Softsoap antibacterial hand soap | Triclosan water, sodium laureth sulfate, cocamidopropyl betaine, decyl glucoside, fragrance, DMDM hydantoin, sodium chloride, PEG 120 methyl glucose diolate, tetrasodium EDTA, sodium sulfate, polyquaternium-7, citric acid, poloxamer 124, PEG 7 glyceryl cocoate, D&C Red No. 33, FD&C Blue No. 1 |
| 94 | Nestle Good Start Supreme DHA and ARA soy infant formula | Corn maltodextrin, vegetable oil, enzymatic hydrolyzed soyprotein isolate, less than 1.5%: sucrose, corn starch, calcium phosphate, potassium citrate, sodium citrate, calcium citrate, <i>M. alpha</i> oil [source of arachidonic acid (ARA)], <i>C. cohnii</i> oil [source of docosahexaenoic acid (DHA)], magnesium chloride, calcium chloride, potassium chloride, ferrous sulfate, zinc 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, thiamin mononitrate, pyridoxine hydrochloride, folic acid, biotin, phylloquinone, vitamin D3, vitamin B12, L-methionine, carrageenan, taurine, L-carnitine |
| 95 | Lipton orange herbal tea | Orange peel, hibiscus flowers, cinnamon, roasted chicory root, clove, licorice root, natural orange flavor (contains soy lecithin) |
| 96 | Mary Kay Timewise night solution | Not available |

stability for crystallization (Pantoliano *et al.*, 2001; Vedadi *et al.*, 2006; Niesen *et al.*, 2007). The assay monitors the fluorescence of SYPRO Orange dye, which produces a signal as it interacts with the hydrophobic residues of a protein. For soluble proteins, hydrophobic residues tend to populate the less solvent-accessible areas, typically residing on the interior of the macromolecule. Upon slow heating, as the macromolecule begins to unfold, these hydrophobic residues are exposed to the dye. The dye interacts with the residues and produces a fluorescence signal that increases as the protein unfolds. The mid-point of this melting curve is defined as the T_m , where the macromolecule is equally distributed between the folded and unfolded states.

Each well of a MicroAmp Fast Optical 96-well reaction plate (4346906, Applied Biosystems, Foster City, California, USA) was filled with 25 μl of deionized water, 1 μl of PSB, 2 μl of 75 μM protein solution (diluted in dd H_2O) and 2 μl of 75X SYPRO Orange dye (S5692, Sigma–Aldrich, St Louis, Missouri, USA). For the proteins lysozyme (C), ovalbumin (K), lactoferrin (P), concanavalin A (Q) and alcohol dehydrogenase (R), 23 μl of deionized water, 1 μl of PSB, 2 μl of dye and 4 μl of protein solution were used to increase the signal. Control experiments, using 1 and 2 μl aliquots of the PSBs with 2 μl of 75X dye diluted to a final volume of 30 μl with dd H_2O , verified that the product stock solutions did not have an effect on the DSF assay (with the exception of PSB 93, hand soap). Reaction components were added to the plate sequentially as described. A multi-channel automated liquid-handling system (801-10005, Thermo Scientific Matrix, Hudson, New Hampshire, USA) or manual multi-channel pipette (L8-50, Rainin Instrument LLC, Oakland, California, USA) was used to dispense the product stock solutions into the reaction plate. A repeater pipette (022260201, Eppendorf North America, Westbury, New York, USA) was used to dispense the water, protein and dye. The plate was sealed with optically transparent heat-resistant film (Hampton Research), mixed by inversion and centrifuged for 5 min at 1000 r min^{-1} . The filled plate was placed into an Mx3005P real-time quantitative PCR system equipped with *MxPro* software for experimental setup, data collection and analysis (401449, Stratagene, Cedar Creek, Texas, USA). The sample was heated at a rate of $\sim 1.5 \text{ K min}^{-1}$ from 298 to 372 K. Fluor-

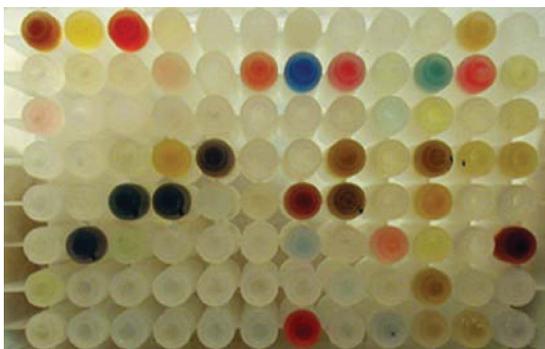


Figure 1

A view from the bottom of the initial 96-solution source or mother plate, showing the colorful nature of the PSBs chosen for the experiment.

escence emission was monitored at 610 nm and recorded at $\sim 0.5 \text{ K}$ increments for each well, after excitation at 492 nm. Upon completion of the experiment, the data were exported to Microsoft *Excel* and then into *GraphPad Prism* (GraphPad Software, San Diego, California, USA) for analysis.

The value of T_m is typically determined by fitting the data to the Boltzmann sigmoidal curve of the data (Niesen *et al.*, 2007). We calculated T_m using two distinct methods with software developed in-house. The first is a nonlinear least-squares fit to the logistics function. The second smoothes the temperature and fluorescence data using a quartic 11-neighbor Savitzky–Golay (SG) filter, and uses the smoothed data as input to compute the first and second filtered derivatives by SG (Savitzky & Golay, 1964). Data analysis to derive the T_m values is fully automated; calculations are completed in minutes, and the processed data are exported to an *Excel* spreadsheet.

The protein reference sample was prepared by diluting a concentrated protein solution with dd H_2O plus the addition of SYPRO Orange dye. In cases where the reference protein provided interpretable melting curves, ΔT_m is defined as $T_m(\text{protein} + \text{additive}) - T_m(\text{reference})$.

This assay requires minimal time and sample. Protein solution requirements are typically 200 μl of 75 mM stock solution to perform this assay for 96 cocktails (150 μg for a 10 kDa protein sample, with increasing amounts required for proteins of higher molecular weight). While it takes 45 min to collect the fluorescence data, it should be noted that human intervention is not required during data collection itself, only to load the samples and analyze the data after they have been automatically processed. The task was accomplished by the students but is routinely run for remote investigators as part of the high-throughput crystallization screening service at HWI.

2.4. Crystallization screening experiments

A modified version of a robotic 1536-well high-throughput microbatch-under-oil crystallization screening protocol was used by the students to set up the crystallization trials (Luft *et al.*, 2003). PEG and buffer stock solutions were prepared at room temperature. 1.0 M buffer stocks were prepared at pH 5.8 [2-(*N*-morpholino)ethanesulfonic acid; MES], pH 6.8 (HEPES) and pH 7.8 (bis-tris propane), using concentrated NaOH or HCl to adjust the pH. A 50% (*w/v*) solution of PEG 3350 was purchased from Hampton Research. Cocktail solutions were prepared by co-dilution of the 1.0 M buffer stock solutions and 50% (*w/v*) PEG 3350 with water to a final concentration of 100 mM buffer and 25% (*w/v*) PEG 3350. The PEG/buffer stock solutions were used to prepare eight different 96-well mother plates of cocktails for the crystallization trials by co-dilution of the $96 \times 5\%$ (*v/v*) and 50% (*v/v*) PSB stock solutions (100% PSB stock diluted with dd H_2O) with an equal volume of 100 mM buffer and 25% (*w/v*) PEG 3350. Final cocktail solutions are described in Table 3.

To set up the crystallization screening experiments, 5 μl of USP grade mineral oil (Sigma–Aldrich) followed by 200 nl of the 8×96 cocktails described in Table 3 were dispensed in

duplicate into 1536-well Greiner microassay plates (Frickenhausen, Germany) using a PlateMate 2 × 2 liquid-handling system (Thermo Scientific Matrix). A 200 nl aliquot of one protein stock solution was dispensed under oil to a single set of the 8 × 96 (768) cocktail solutions, followed by a wash cycle and then delivery of a second protein stock solution to the second set of 768 cocktails. Each 1536-well plate held two different proteins combined with the 768 cocktails. After protein delivery had been completed, the plate was centrifuged at low speed (1000 r min⁻¹, 3 min) to ensure that the protein and cocktail drops merged together under the oil and were resting against the bottom of the well for imaging. Digital images of the experimental outcomes were recorded at room temperature using a custom-designed robotic imaging system (Fig. 2) at time zero (immediately after centrifugation). Plates were then incubated at 296 K and removed for imaging at room temperature at 1 d and one, two, three and four weeks after time zero for all 20 proteins × 768 cocktails.

The digital images were examined using an in-house program called *MACROSCOPE* (Luft *et al.*, 2003). This program displays 96 images at a time and allows them to be classified in either single (Snell, Lauricella *et al.*, 2008) or multiple categories of outcomes, *e.g.* clear drop, phase separation, crystal or precipitate (Snell, Luft *et al.*, 2008). For our purposes, the images were examined after each read and classified in a binary fashion, crystals or no crystals. The resultant classifications were analyzed.

As in the DSF study, controls are an essential part of the scientific method. We conducted these on a number of levels. The 20 proteins were set up with Hampton Research Silver



Figure 2

The robotic plate-imaging system in operation. A total of 28 plates can be placed on the translation stage at any one time, with the images being made available *via* a secure ftp site as soon as an individual plate has been completed. The process is part of the high-throughput crystallization screening pipeline at HWI and operates completely automatically, requiring only placement of the 1536-well plate on the translation stage where the position is automatically indexed. Chemical data are linked to the images through a database. One of the students involved with the project, Nicholas Furlani, is shown placing a plate on the imaging robot. In a more accessible application of this program, students would not have to be physically present in the HWI laboratory, as the robotic processes and data dissemination would allow the students to gain access to the image data from remote locations.

Table 3

The 8 × 96 crystallization cocktail solutions.

Each PSB is set up in solutions A to H. This produces 768 different conditions and allows two proteins to be set up in a single 1536-well plate.

| | Product stock concentration (% v/v) | Buffer | pH | PEG 3350 (% w/v) |
|---|-------------------------------------|------------------------|-----|------------------|
| 1 | 50.0 | Unbuffered | | |
| 2 | 5.0 | Unbuffered | | |
| 3 | 25.0 | 50 mM MES | 5.8 | 12.5 |
| 4 | 25.0 | 50 mM HEPES | 6.8 | 12.5 |
| 5 | 25.0 | 50 mM bis-tris propane | 7.8 | 12.5 |
| 6 | 2.5 | 50 mM MES | 5.8 | 12.5 |
| 7 | 2.5 | 50 mM HEPES | 6.8 | 12.5 |
| 8 | 2.5 | 50 mM bis-tris propane | 7.8 | 12.5 |

Bullets, a carefully designed and influential screen where the chemical additives were chosen based on the hypothesis that small molecules could be used to establish stabilizing intermolecular noncovalent crosslinks in protein crystals to promote lattice formation, as a benchmark of effectiveness. To avoid false positives (crystals that were not protein, but rather artefacts of interactions between the product stock solutions and crystallization cocktails), we delivered 200 nl of dd H₂O to the cocktails instead of protein solutions as one control, and 200 nl of protein buffer to the cocktails instead of protein solutions as a second control. Finally, the proteins themselves were set up with the stock solutions in Table 3 not containing the PSBs, to determine if the resulting crystals would form in the absence of a PSB.

3. Results

3.1. Differential scanning fluorimetry

Examples of the DSF data are given in Fig. 3. The data show typical Boltzmann sigmoidal melting curves for protein Q, concanavalin A. The fluorescence counts remain stable as the temperature increases until the protein begins to unfold. When the SYPRO Orange dye begins to interact with the hydrophobic interior residues of the protein, which become solvent-accessible as the protein unfolds, the dye begins to fluoresce. The fluorescence signal continues to increase as the protein is heated and continues to unfold, exposing more of the hydrophobic interior residues. At the inflection point, the protein is considered to be half folded and half unfolded. The inflection point value is taken as the melting temperature (T_m) of the protein. In Fig. 3, DSF data for the protein concanavalin A are displayed as three distinct melting curves. The control, with a T_m value of 342.9 K, is the protein in buffer with no PSB added. The melting temperature of the control falls between those for the same protein and buffer solutions with the addition of a stabilizing PSB, Afta aftershave lotion ($T_m = 355.1$ K), which increases the melting temperature compared with the control ($\Delta T = 12.2$ K), and in the presence of a destabilizing PSB, Pez Cherry candy ($T_m = 331.7$ K), which decreases the melting temperature compared with the control ($\Delta T = -11.2$ K).

The complete DSF results are tabulated in Fig. 4. Only 11 of the 20 proteins gave useable DSF signals. The remainder had

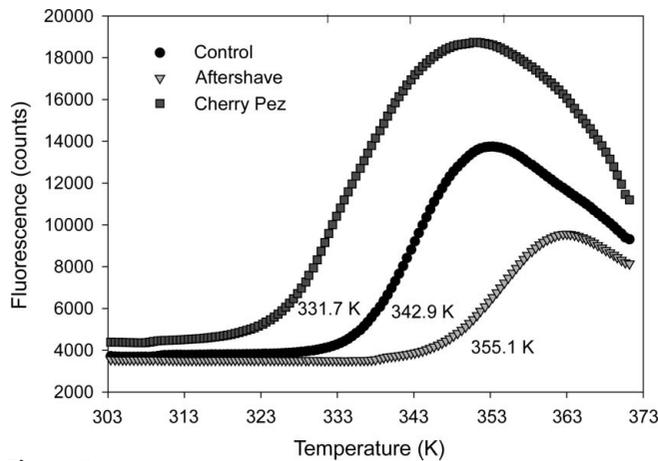


Figure 3
DSF results for concanavalin A. The melting temperature is obtained from the inflection point of the initial rise in fluorescence. Three curves are shown, a control curve with a melting temperature determined to be 342.9 K and then curves for two PSBs, aftershave and Cherry Pez, with melting temperatures of 355.1 and 331.7 K, respectively.

| No. | Pseudo silver bullet (PSB) | C | G | E | H | K | O | P | Q | R | S | T |
|-----|----------------------------|------|-------|-------|-------|-------|-------|-------|-------|------|-------|-------|
| 1 | Bubbles | -0.5 | -0.1 | -0.2 | | -1.0 | -0.1 | -2.6 | -8.4 | | -0.7 | -1.4 |
| 2 | Distilled white vinegar | | -2.0 | -0.1 | | -10.5 | | -20.4 | 4.7 | | | -10.8 |
| 3 | Clorox disinfect cleaner | -6.0 | -11.2 | -10.2 | | | -2.3 | -3.8 | 10.3 | | | -9.5 |
| 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 |
| 5 | Dove hairspray | | | | | | | -1.3 | 2.8 | -2.6 | | |
| 6 | Scope mint mouthwash | 1.6 | 0.0 | 0.1 | | -1.2 | 0.2 | -0.3 | 14.1 | -1.9 | 1.0 | -1.8 |
| 7 | Nyquil Liquid- cherry | | | | | -0.4 | 0.6 | 5.2 | -19.6 | -1.0 | 2.7 | 1.7 |
| 8 | Proactive Revitalize toner | | | | -0.6 | -11.1 | | -21.2 | 8.1 | | | -10.6 |
| 9 | Sudafed PE children's | 4.5 | 0.5 | 3.2 | | 0.1 | -2.3 | 3.6 | 2.4 | -1.2 | -0.3 | 1.1 |
| 10 | Red wine vinegar | | -1.5 | 0.8 | -0.3 | -10.5 | | -16.3 | 7.7 | | | -16.4 |
| 11 | Coke Cola, diet | 4.9 | 0.5 | -3.3 | | 0.5 | -0.1 | 3.2 | 11.3 | 0.3 | -3.3 | -1.0 |
| 12 | Listerine fluoride rinse | | -12.1 | -2.5 | | -0.8 | 0.0 | -1.0 | 24.8 | -6.3 | -2.1 | -2.5 |
| 13 | Dill spears juice | 4.6 | 0.1 | 5.4 | 0.9 | -4.4 | | -3.8 | 2.0 | 6.1 | -7.0 | 1.3 |
| 14 | Diet peach grapefruit | 4.6 | 0.1 | 4.0 | | 0.0 | -0.3 | 4.4 | 1.4 | -1.1 | 0.5 | 0.6 |
| 15 | Heinz cleaning vinegar | 3.9 | -2.3 | -0.1 | -1.0 | -12.0 | | -22.2 | 2.5 | | | -10.4 |
| 16 | Febreze fabric refresher | | -5.0 | -0.4 | -7.1 | 0.2 | 1.0 | 5.8 | 9.7 | -0.3 | 0.8 | 0.9 |
| 17 | Contact solution | 2.4 | -0.1 | 0.1 | | -0.8 | -0.2 | 0.2 | -0.2 | -0.2 | -0.7 | 0.3 |
| 18 | Hydrogen peroxide | 0.2 | 0.0 | 0.3 | | -0.8 | -1.7 | 0.8 | -33.2 | -2.9 | -0.6 | -0.5 |
| 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 |
| 20 | Deep-cleaning astringent | | | | | -1.2 | -5.7 | 4.0 | 5.9 | -3.3 | -5.1 | |
| 21 | Margarita mix | 0.9 | -11.7 | -13.4 | | -12.2 | | -11.8 | 1.9 | | | -15.6 |
| 22 | Tiparos fish sauce | 3.5 | 1.9 | 1.5 | | 0.2 | -1.0 | 5.5 | -2.1 | -0.4 | -1.1 | -1.2 |
| 23 | Body spray, vanilla | | | | | 0.3 | 0.9 | -0.4 | -3.2 | -2.9 | | |
| 24 | Bathroom cleaner | | | | -1.5 | -14.4 | | -22.7 | 2.6 | | | -9.1 |
| 25 | Polish remover, regular | 0.3 | -0.1 | 0.3 | | -0.8 | 0.2 | -0.5 | -1.0 | -0.2 | 0.3 | -0.2 |
| 26 | Red food coloring | 2.8 | | | | | | | | -0.7 | | |
| 27 | 2-in-1 body shampoo | | | | | | | | | -0.9 | | |
| 28 | Gel dishwash detergent | 5.5 | | | | | | | | 1.0 | | |
| 29 | Purell hand sanitizer | -7.9 | -0.1 | -9.3 | | 0.0 | 0.4 | 2.0 | 2.1 | -2.5 | -0.2 | -6.1 |
| 30 | Airborne baby wash | | | | | | | | | -0.4 | | |
| 31 | Dawn dishwash liquid | | | | | | | | | -1.2 | | |
| 32 | Excedrin | -8.6 | -2.7 | 0.3 | 0.0 | -8.5 | | -14.1 | -2.3 | | | -17.7 |
| 33 | Cinnamon mint | 1.6 | 0.4 | 0.3 | | 0.1 | 0.7 | 1.3 | 4.2 | -3.1 | 0.9 | -0.8 |
| 34 | Wegmans dish detergent | | | | | | | | | -4.0 | | |
| 35 | Baking soda | 2.2 | -5.0 | -13.9 | | -2.7 | -16.6 | 3.7 | 11.2 | -8.5 | -14.2 | -15.8 |
| 36 | Cranberry pomegranate | | | | | | | | | 3.0 | | |
| 37 | Tilex mildew remover | | -9.4 | -13.6 | | | | -5.9 | | 8.7 | | -15.3 |
| 38 | Lemon juice | | -4.2 | -7.2 | 0.2 | -14.4 | | -20.0 | 13.1 | | | -25.5 |
| 39 | Maple extract | | | | | | | | | 15.0 | | |
| 40 | Balsamic vinegar | | -2.2 | | | | | -12.5 | 4.1 | | | -14.6 |
| 41 | Afta aftershave lotion | | -2.5 | -2.7 | | 0.1 | -6.8 | | 12.2 | 13.5 | -20.8 | 0.9 |
| 42 | Heinz tomato ketchup | 2.9 | -4.4 | 2.5 | 0.4 | -4.4 | -20.9 | -0.2 | -0.5 | 4.1 | -4.9 | -4.8 |
| 43 | Raspberry lemonade mix | | -7.4 | -11.6 | -6.0 | -15.6 | | | 6.7 | | | -1.8 |
| 44 | Ground black pepper | 2.8 | 0.7 | -1.4 | | -0.2 | 1.1 | 2.7 | 12.1 | -0.1 | 1.2 | 5.8 |
| 45 | Powder dish detergent | | -2.3 | -8.3 | -20.7 | | -7.9 | | -3.2 | 11.6 | | -20.4 |
| 46 | Ground cinnamon | -2.8 | -0.2 | 1.7 | | 0.4 | 1.3 | 2.0 | -1.9 | 15.3 | 6.3 | 0.0 |
| 47 | Wegmans salt, iodized | 1.1 | 0.0 | 0.8 | | 0.1 | 0.5 | 4.5 | 2.7 | 12.1 | -1.1 | -3.1 |
| 48 | Avon loose powder | 0.0 | 0.4 | 0.1 | | 0.3 | 0.8 | 0.8 | | -0.6 | -0.4 | -0.9 |

Figure 4
A table of DSF results for the proteins and PSBs. The numbers represent temperature shifts in K. Only 11 of the 20 protein samples gave useful DSF signals. PSB 93, hand soap, was removed as it was hydrophobic and significantly interfered with the DSF assay by interacting with the dye and causing unacceptably high background fluorescence, making the data uninterpretable. We used this position as a control, with only the protein and dye diluted with water loaded in that well. The temperature shift was calculated by reference to this control. In the case of proteins H, R and S a melting temperature could not be calculated from the control data. Blank areas in the table indicate that the melting data were difficult to impossible to interpret. In cases H, R and S, where we could not calculate a melting temperature of the protein in the absence of a PSB, the values in the table were calculated as a shift from the average value of the T_m for that protein for all of the interpretable T_m values of the protein with a PSB. Green and pink shaded cells denote temperature shifts $\geq \pm 2.0$ K, respectively.

either high background fluorescence readings (caused by interactions between the SYPRO Orange Dye and exposed hydrophobic residues of the protein, or a hydrophobic solution environment caused by hydrophobic PSBs) or uninterpretable melting curves which did not have the typical profile of a Boltzmann sigmoidal curve. It is interesting to note that alcohol dehydrogenase and chymotrypsin only provided an interpretable melting curve after the addition of specific PSBs. This could be interpreted as a structural change in these proteins caused by a particular small molecule from the PSB cocktail that reduced the exposed hydrophobic surface of the protein.

The PSBs had mixed performances on stabilizing the proteins (*i.e.* raising the melting temperature). We considered the DSF data to be significant when the melting temperature increase or decrease was ≥ 2 K. There were seven PSBs that increased the melting temperature for four out of 11 proteins that had interpretable DSF data. These additives were Sudafed (9), ground black pepper (44), prenatal vitamins (56),

| No. | Pseudo silver bullet (PSB) | C | G | E | H | K | O | P | Q | R | S | T |
|-----|----------------------------|-------|-------|-------|-------|-------|-------|-------|-------|------|-------|-------|
| 49 | Cascade dish detergent | -4.3 | -7.9 | -21.9 | | -8.3 | | -4.5 | 2.0 | | -22.8 | |
| 50 | Equal sweetener | 2.3 | 0.4 | 1.1 | | -0.2 | -0.3 | 0.2 | 0.2 | -0.6 | 0.8 | 1.5 |
| 51 | Severe cold, daytime | | -3.2 | -1.6 | -1.0 | -10.3 | | -10.0 | 7.7 | | | -16.0 |
| 52 | Centrum multivitamin | 2.6 | 1.1 | 2.6 | | -1.4 | -1.5 | 28.2 | | -3.9 | 1.0 | -1.2 |
| 53 | Folgers roast coffee | | -2.5 | | | -0.2 | 0.4 | 8.6 | 7.9 | -1.4 | 6.3 | |
| 54 | Gerber cherry puffs | 0.3 | 0.2 | | | -0.1 | 0.5 | 0.7 | -5.9 | 0.1 | 0.0 | -0.7 |
| 55 | Ibuprofen | -5.0 | 0.1 | -3.3 | | 0.0 | 0.7 | -0.2 | 2.8 | -0.8 | 0.0 | -0.6 |
| 56 | Prenatal vitamins | 1.6 | 0.3 | | | -1.0 | -0.4 | 23.7 | 5.2 | 10.4 | 5.2 | -0.3 |
| 57 | Halls defense | | -0.8 | | 0.9 | -7.4 | | -3.0 | 2.1 | | -11.0 | |
| 58 | Women's vitamins | 0.7 | -0.2 | 3.3 | | -1.1 | -1.9 | 30.2 | | 4.6 | 1.4 | 2.1 |
| 59 | Wegman's honey | 4.8 | 0.2 | 4.3 | | 0.3 | -0.4 | 2.5 | -5.6 | 0.1 | 2.9 | 2.0 |
| 60 | Spices | 2.9 | -2.2 | 1.6 | | 0.2 | 0.1 | 4.5 | -25.5 | -0.5 | 0.9 | 8.5 |
| 61 | Children's Thin Strips | -1.4 | 0.5 | 0.8 | | -1.0 | 0.5 | 0.5 | | -0.5 | -4.3 | -1.8 |
| 62 | MK Timewise day | -6.7 | -5.2 | -10.2 | 5.2 | -4.8 | -0.3 | 5.2 | | 1.6 | -5.0 | -2.8 |
| 63 | MK Timewise 3-in-1 | | | | | -7.7 | 0.2 | 0.2 | 0.9 | | -0.4 | -1.6 |
| 64 | MK TW step 2: replenish | -3.2 | -16.4 | -7.6 | 5.0 | 0.0 | -1.9 | 0.5 | 2.3 | 0.0 | -0.1 | 1.1 |
| 65 | Rubbing alcohol | -0.4 | -1.7 | -0.3 | | -3.3 | -0.5 | -1.4 | -4.4 | -1.4 | -0.7 | -0.9 |
| 66 | Clorox sanitizing spray | | -2.0 | -0.1 | 0.2 | -0.2 | -0.2 | 0.3 | | -0.2 | 0.2 | -0.1 |
| 67 | Ron Bacardi rum | -0.3 | -0.3 | -0.2 | | -1.2 | -0.4 | -0.4 | | -0.6 | -0.2 | 0.2 |
| 68 | Golden Fields fiber | 2.2 | -2.8 | -0.7 | | -0.1 | -0.1 | 2.5 | -4.2 | 0.2 | 0.1 | 0.0 |
| 69 | Windshield washer fluid | 0.0 | 0.0 | -0.1 | | -0.6 | 0.2 | -0.6 | | 0.8 | 0.3 | -1.2 |
| 70 | Lemon Lime Gatorade | | -1.3 | 3.6 | | -5.2 | | 1.8 | | 1.5 | -9.7 | 1.7 |
| 71 | Rite-Aid aspirin | -4.2 | -2.0 | 1.9 | | -4.4 | | -3.5 | | 5.8 | -11.1 | 2.7 |
| 72 | Chiavetta marinade BBQ | -1.5 | -4.1 | -3.8 | -0.7 | -12.2 | | -16.7 | 7.0 | | | -21.1 |
| 73 | Splenda sweetener | 0.5 | 0.3 | 0.1 | | 0.3 | 0.0 | 2.2 | -3.6 | -0.8 | 0.2 | -0.4 |
| 74 | Sugarfree Redbull | | -0.9 | | 0.3 | -4.0 | | 3.5 | 3.5 | 6.6 | -6.4 | 3.0 |
| 75 | Monster Energy | | -0.3 | | | -1.4 | | 5.0 | 11.3 | 1.2 | -6.0 | 0.7 |
| 76 | Tums cool relief | -0.7 | 0.6 | -0.3 | | -0.1 | 1.1 | 3.3 | -6.3 | -0.1 | -1.5 | -1.7 |
| 77 | Sugar, white, granulated | 0.4 | 0.2 | 0.1 | | 0.0 | 0.2 | 1.2 | -4.3 | -0.1 | 0.2 | 0.1 |
| 78 | Nesquik strawberry | 0.0 | 0.4 | 0.1 | | -0.1 | 0.6 | 4.5 | 10.7 | 0.7 | 3.0 | 0.4 |
| 79 | Fizzy Tub Colors | 11.7 | -2.4 | -8.0 | | -0.4 | -2.4 | 11.2 | -5.0 | -3.1 | -9.3 | -8.7 |
| 80 | L'Oréal mineral blush | 0.5 | 0.2 | -0.5 | | 0.1 | 1.4 | 1.6 | -0.8 | 0.4 | -0.8 | -2.5 |
| 81 | Tums antacid | -1.5 | 1.1 | -0.3 | | -0.4 | 0.9 | 2.6 | -4.4 | 0.3 | -0.6 | -3.4 |
| 82 | Cake Mate sprinkles | 1.2 | 0.2 | 0.9 | | 0.2 | 0.8 | 0.6 | -4.8 | 0.1 | 1.0 | -0.1 |
| 83 | Tylenol cold, daytime | 0.2 | 0.2 | 0.4 | | -0.9 | 0.5 | 0.2 | -5.5 | -1.3 | -1.2 | -0.5 |
| 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 |
| 85 | Cold-Eeze lozenges | | 0.3 | 5.2 | 0.6 | -6.0 | -16.2 | -1.5 | -1.2 | | | |
| 86 | Rolaids antacid, cherry | -0.4 | 1.2 | 0.1 | | -0.4 | 0.1 | 3.2 | 4.1 | -0.2 | -1.7 | -4.6 |
| 87 | Raspberry coffee syrup | | -0.2 | 4.9 | | -0.3 | -2.6 | 6.1 | -4.6 | -1.9 | 4.6 | 1.9 |
| 88 | Pez, cherry | 3.2 | 0.3 | 0.4 | | 0.0 | -0.1 | 1.1 | -11.2 | -0.2 | 0.0 | 0.0 |
| 89 | Infants' antias, drops | 1.8 | -7.7 | -2.3 | -15.5 | -0.2 | 0.9 | 3.4 | -4.7 | -0.2 | 0.1 | -0.6 |
| 90 | Coconut conditioner | -11.7 | -0.3 | -1.8 | 6.7 | | -0.1 | -3.8 | 0.2 | | -8.6 | -3.3 |
| 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 |
| 92 | Airborne hand lotion | -4.6 | -0.3 | 0.1 | | 0.0 | 0.2 | 0.4 | | -0.5 | 0.3 | 0.0 |
| | Control point | 0.0 | 0.0 | 0.0 | | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 94 | Good Start supreme | 2.0 | 0.9 | 1.8 | | -0.3 | 0.1 | 3.3 | -16.4 | 0.4 | 3.7 | -0.4 |
| 95 | Lipton orange tea | -0.9 | -3.5 | -1.2 | | -0.1 | -1.2 | 2.5 | -5.5 | -0.5 | -6.3 | 0.8 |
| 96 | MK Timewise night | -5.5 | -4.4 | -9.8 | 5.9 | 0.3 | -0.5 | 0.8 | 0.9 | -4.2 | | -0.8 |

women's vitamins (58), honey (59), Sugarfree Redbull (74) and mustard (91). One has to be careful in the interpretation of these data as the PSBs may be causing aggregation of the protein, rather than having a stabilizing effect on the protein to raise the T_m . This could be investigated further using dynamic light scattering to study the size and polydispersity of the proteins in the absence and presence of the PSB, but such an investigation was not undertaken as part of this study. Four PSBs destabilized the proteins in a number of cases. Margarita mix (21), baking soda (35), lemon juice (38) and Fizzy Tub Colors (79) decreased the T_m value for at least six out of 11 proteins. Decreases in the melting temperature could be due to the PSB helping the protein to unfold or denature partially, causing the hydrophobic interior regions of the protein to be exposed to the fluorescent dye at a lower temperature.

3.2. Crystallization screening experiments

The controls proved to be a critical component of these experiments. For the 20 proteins studied, three crystal hits were observed in H₂O alone [cytochrome *c* (B), bovine hemoglobin (L) and lactoferrin (P)]. For the proteins in pH 5.8 buffer, two hits were seen [for creatine kinase (O) and lactoferrin (P)], with two for pH 6.8 [proteinase K (J) and bovine hemoglobin (L)] and finally three for pH 7.8 [lipase (I), hemocyanin (N) and lactoferrin (P)]. When the PSBs were present, only in the case of bovine hemoglobin (L) was a crystal hit identified in the PSB with H₂O alone. Creatine kinase (O) showed two crystal hits at pH 5.8 and lactoferrin (P) showed one hit with the PSBs present. At pH 6.8, many hits were seen for proteinase K (J) and none for bovine hemoglobin (L) with the PSBs present. Finally, for pH 7.8, lipase (I) showed one hit, hemocyanin (N) two hits and lactoferrin (P) no hits.

The second set of controls was conducted with the PSBs in water and buffer and no protein. Possible crystal hits occurred in 14 cocktails with water alone. These were with PSBs 4, 22, 27, 28, 30, 34, 41, 53, 56, 58, 59, 63, 84 and 91. For the PSBs in buffer, crystal hits were seen in many of the cocktails. Eight of the nine cocktails with PSBs 60 and 85 showed crystal hits, seven for PSBs 13, 57 and 95, and six for PSBs 20, 22, 41, 43, 48, 51, 53, 56, 58, 59, 68 and 91. Five hits were seen for 11 PSBs, four hits for 12, three hits for 14, two hits for 19, one hit for 14 and no hits for the remaining 11 PSBs. For the control case, the classification of the results erred on the side of caution, with anything seemingly close to a crystal hit being classified as such. In cases where we observed hits with the PSBs in the absence of protein, we were crystallizing the PSB. As a worst-case scenario, we focused on a single pH (6.8) and concentration [25% PSB (*v/v*)]. Out of 247 hits for all 20 proteins, 194 (78%) were unique to the PSB/protein combination and could not be explained by the PSB alone, owing to their absence in the control experiment. Examining the complete set of results (Fig. 5), it becomes apparent that the real protein crystal results are a much higher percentage than this. In a majority of cases, 13 out of 20 proteins, few crystal hits result and all are from different PSBs. If we were indeed crystallizing the PSB

alone we would expect to see overlap with many common results, rather than the non-overlapping hits we observe.

In Fig. 6, a graph is shown of the number of crystal hits for all 20 proteins four weeks after initial setup *versus* the type of PSB. PSBs with 29 or more hits are labeled. The minimum number of hits was five, for PSB 27 (2-in-1 body shampoo) and PSB 36 (cranberry pomegranate mix), followed by PSBs 26 and 44 (red food coloring and pepper). It is notable that of the seven products having the highest number of hits, four were over-the-counter (non-prescription) pharmaceuticals. Other over-the-counter pharmaceuticals, *e.g.* PSBs 7, 9, 32, 51 and 85, had average performances.

Fig. 7 shows the number of hits as a function of protein. Several proteins had a large number of hits, such as concanavalin A and catalase with over 400, and urease with almost 400. A larger number of proteins showed few hits, *e.g.* bovine hemoglobin, ovalbumin, lactoferrin, lipase, thaumatin,

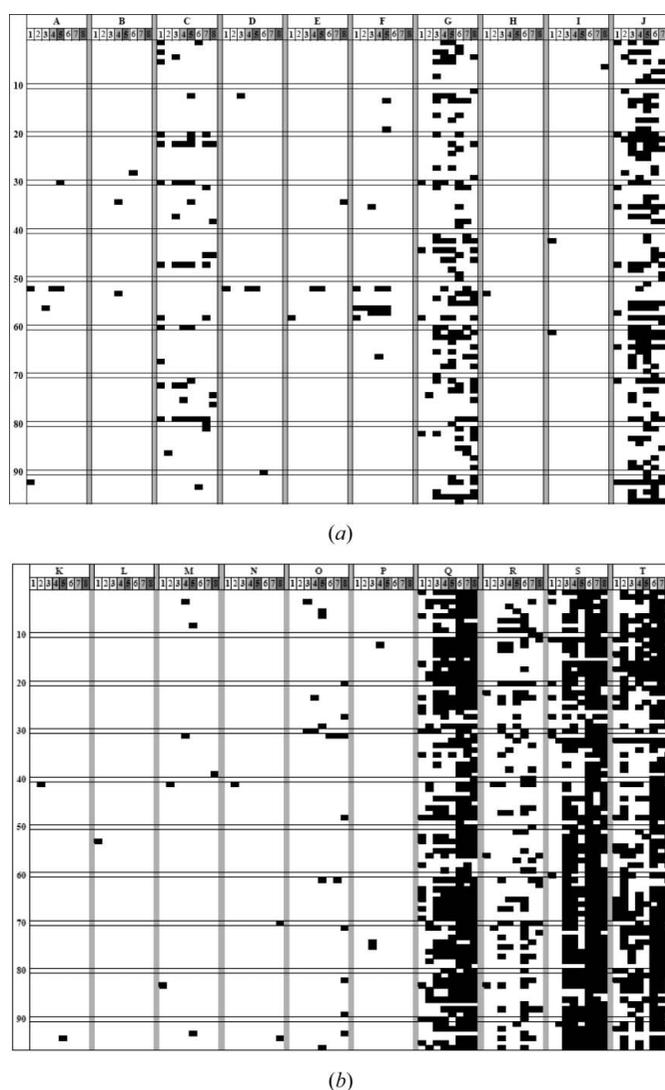


Figure 5 Crystal hits from the PSB cocktails. The proteins are arranged (a) A–J and (b) K–T. There are eight columns for each protein, representing the cocktails 1–8 (Table 3), and 96 rows, representing the 96 PSBs. Crystallization hits are represented by filled blocks. Clear blocks represent null results.

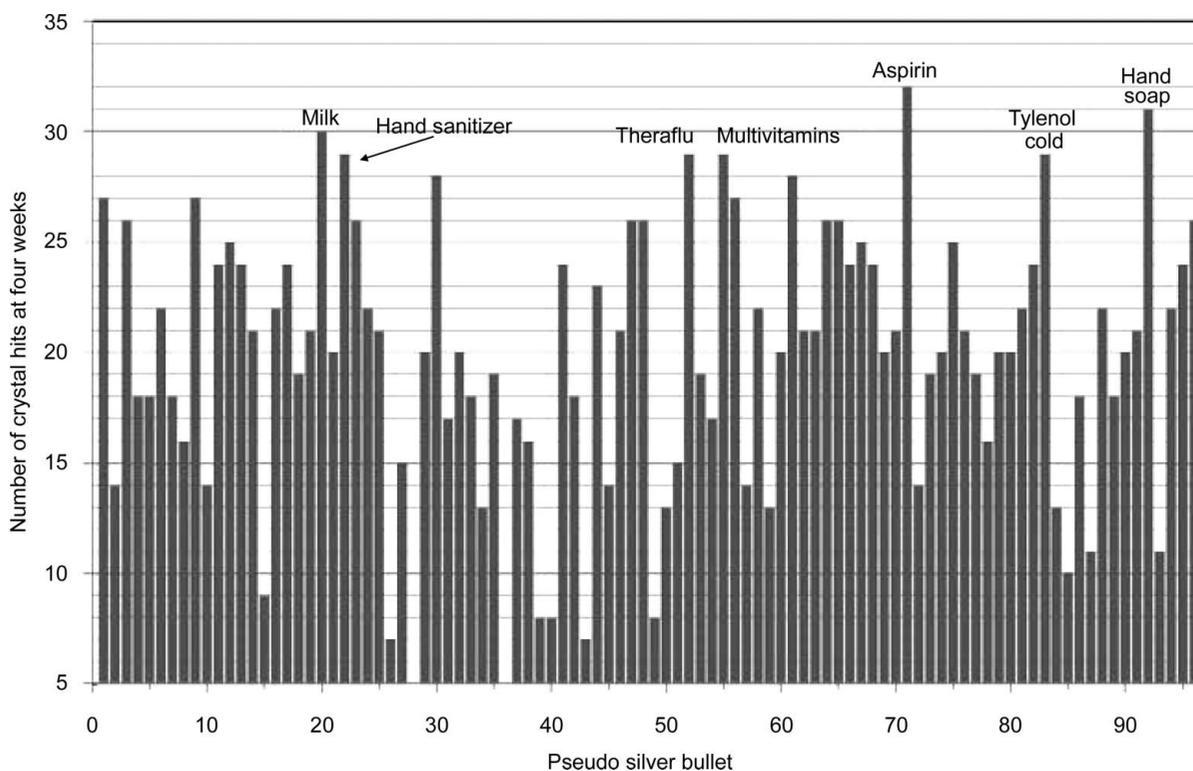


Figure 6

The number of crystal hits for all 20 proteins, plotted against the PSBs given in Table 2. All PSBs had a minimum of five hits. For those with 29 hits or more the PSB is identified on the graph.

chymotrypsin, cytochrome *c* and hemocyanin each had less than five hits. This is not unexpected given the hypothesis surrounding the use of 'silver bullets' as a crystallization mechanism, requiring protein-specific interactions. For these proteins the PSBs that gave hits are listed in Fig. 4. Our control data showed hits for five of the 11 proteins in this table under conditions where the PSB was not present. Out of these, there

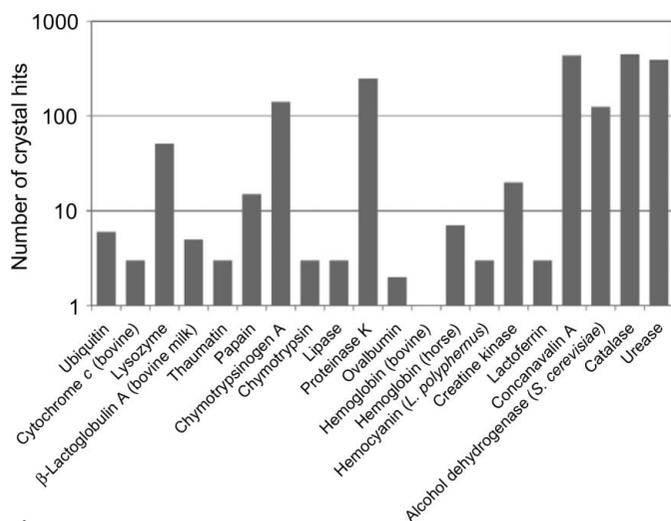


Figure 7

The number of hits shown as a function of protein. For some proteins there were a large number of hits, the largest being bovine liver catalase with 434. For others there were few, bovine hemoglobin showing only one. For this reason the data are presented on a logarithmic scale.

was only a single hit for each of proteins I, N and P that could be explained in the absence of the PSB.

In Fig. 8, the number of crystallization hits is plotted against the PSB formulation, *i.e.* 50% (v/v), 5.0% (v/v), and both 25% (v/v) and 2.5% (v/v), in pH 5.8, 6.8 and 7.8 buffers. This shows the compiled results for all 20 protein samples. For those with few hits (20 or fewer) there was no clear trend identified as the data were statistically unreliable. For those with more than 20 hits, *i.e.* catalase, urease, creatine kinase, alcohol dehydrogenase, chymotrypsinogen A, concanavalin A, and proteinase K, the individual trends were similar to the

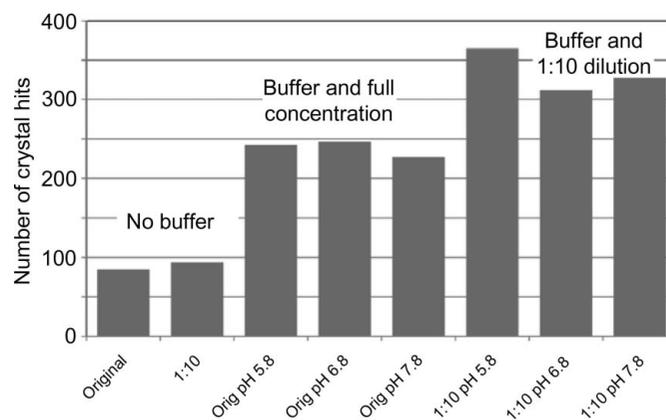


Figure 8

A plot of the number of crystallization hits against the conditions in which the experiments were set up, *i.e.* the original concentration of the PSB and 1:10 dilution, then the original and diluted PSB at three different pH values, 5.8, 6.8 and 7.8.

overall trend. Samples in buffered 2.5% (v/v) PSB cocktails showed more crystal hits than the unbuffered 2.5% (v/v) and 25% (v/v) PSB cocktails and the buffered 25% (v/v) PSB cocktails. The exception to this trend was lysozyme, protein C. In this case most hits were seen in the unbuffered 25% (v/v) PSB conditions. Fewer hits were seen in the buffered and unbuffered 2.5% (v/v) PSB cocktails. This may not be such a surprise as the lysozyme was set up for crystallization screening at significantly higher concentration than the other protein samples (Table 1).

In comparing individual proteins, we will consider first those that did not provide DSF data and then those that did. Ubiquitin (A), had four hits in baby wash (30), multivitamins (52), prenatal vitamins (56) and hand lotion (92). Ubiquitin acts as a signaling protein by covalent attachment to other proteins. The baby wash and hand soap suggest a common crystallization promoter, although ingredients for the former are not available. Similarly, the vitamin hit suggests a PSB crystallization mechanism, supported by the low number of overall hits – typical of the silver bullet approach. Cytochrome *c* (B) showed hits in three PSBs, gel dishwashing detergent (28), dish detergent (34) and coffee (53). Cytochrome *c* is an electron-transfer protein. It is not immediately obvious to us why these combinations of PSBs should lead to crystallization.

β -Lactoglobulin A (B) gave hits in Listerine (12), multivitamins (52) and coconut conditioner (90). Again, it was not immediately obvious if there was a common chemical ingredient leading to this effect, or a combination of protein-specific effects in some cases and non-specific chemical properties of the solutions in others. Papain (F) gave hits in the PSBs dill spears juice (13), fat-free milk (19), baking soda (35), multivitamins (52), coffee (53) and cherry puffs (54). Lipase (I) gave hits in Scope mouthwash (6), tomato ketchup (42) and Children's Thin Strips (61). Lipase catalyzes the hydrolysis of ester chemical bonds in water-insoluble lipid substrates. One might expect to find these in ketchup and possibly in the other two PSBs.

Proteinase K (J) had many hits and it was not possible to identify any specific PSBs that might provide a positive crystallization effect. At the opposite extreme, bovine hemoglobin (L) only had a single hit in PSB 53, coffee in unbuffered dd H₂O. Horse hemoglobin (M) had multiple hits in Clorox (3), Proactive toner (8), dishwashing liquid (31), maple extract (39), aftershave (41), Tylenol Cold (83) and hand soap (93). Finally, hemocyanin (N) had hits in aftershave (41), Gatorade (70) and infant formula (94). Both the aftershave and Gatorade contain a yellow food dye, tartrazine. This dye is not listed in the infant formula ingredients and is unlikely to be present as it is required to be declared by the FDA. However, there are a number of small molecules that could easily perform chemically similar functions in the ingredients of the infant formula.

Of note was the comparison run between the students' PSBs and the commercially available Silver Bullets. A striking result was observed for Proteinase K, which crystallized in the presence of fish sauce (Fig. 9*a*). This closely matched the

crystal outcome from Silver Bullet H12, which contains aspartame, gly-gly-gly, leu-gly-gly, pentaglycine, tyr-ala and tyr-phe (Fig. 9*b*). With hindsight this does not come as a great surprise, given that fish sauce is made from fermented anchovies and therefore contains peptides, short amino acid chains. In this case the PSB is acting in a manner similar to that of the Silver Bullets, with the added benefit of also providing the remaining bottle of fish sauce for other uses.

3.3. Lessons from the combination of DSF and crystallization

The most interesting results came from the 11 proteins where DSF data were available. The PSBs had mixed performances on affecting the melting temperature as determined from DSF (Fig. 4). For protein C, lysozyme, the most significant temperature increases resulted from Diet Coke (4.9 K), dishwashing detergent (5.5 K) and Fizzy Tub Colors (11.7 K). It crystallized in a number of PSBs but of particular note were 22, 47 and 79, fish sauce, salt and Fizzy Tub Colors. In these cases crystallization took place in multiple formulations of the PSB. We suspect that, rather than acting as a silver bullet crystallization mechanism, the increased salt concentration has reduced solubility for this naturally easy to crystallize protein.

For protein E, thaumatin, the most significant temperature increases resulted from grape juice (5.5 K), dill juice (5.4 K) and milk (5.5 K). Positive temperature increases were also seen for multivitamins and women's vitamins. Thaumatin is a plant protein that is about 100 000 times sweeter than sucrose. Incorporation of tartrate into the crystallization mix induced crystal contacts that enabled crystallization of three different crystal forms (Ko *et al.*, 1994). The chemical structure of tartrate is shown in Fig. 10(*a*), with that of ferrous fumarate, an ingredient in the multivitamins, in Fig. 10(*b*). There is chemical similarity and we suspect that this may be a key element in the increase in protein stability. We do not know the ingredients for the women's vitamins, and the prenatal vitamins did not provide useful DSF data for this protein. Numerous other small molecules are present in the multivitamins. Crystallization

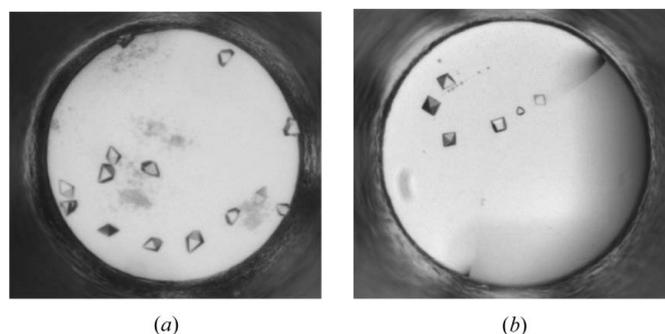


Figure 9
Crystallization results from proteinase K (*a*) from fish sauce with 12.5% PEG 3350 in HEPES buffer at pH 6.8, and (*b*) using Hampton Research Silver Bullet H12 with 12.5% PEG 3350 in HEPES buffer at pH 6.8. Silver Bullet H12 contains aspartame, gly-gly-gly, leu-gly-gly, pentaglycine, tyr-ala and tyr-phe, which are also likely to be found in fish sauce made from fermented anchovies. The well diameter is 0.9 mm.

lization hits were seen with dish detergent (34), multivitamins (52) and women's vitamins (58). While we puzzled over the first (which gave no useable DSF data), the last two are consistent with silver bullet effects and showed a small (within the limits of noise) rise in the melting temperature.

For chymotrypsinogen A (G), no increase in melting temperature was seen but many crystal hits were identified. Chymotrypsinogen A is an inactive precursor of a digestive enzyme; trypsin cleavage is required to form the active enzyme, chymotrypsin, which preferentially catalyzes the hydrolysis of peptide bonds involving L-isomers of tyrosine, phenylalanine and tryptophan, and readily acts upon amides and esters of susceptible amino acids. Chymotrypsin (H) showed a melting temperature increase in PSBs 62, 64, 90 and 96. With the exception of PSB 90, coconut conditioner, these PSBs are all Mary Kay products where no ingredient information is available. Only a single crystal hit was seen for this protein in PSB 53, coffee.

The proteins ovalbumin (K) and creatine kinase (O) showed no significant melting temperature increase with the PSBs. Ovalbumin is the main protein found in egg white and is thought to be a storage protein. Crystallization hits occurred in PSBs 41 and 94, Afta aftershave lotion and infant formula. Ovalbumin is purified by crystallization (Judge *et al.*, 1995) using ammonium sulfate, with pH influencing the resultant crystal volume. The infant formula contains a number of sulfate salts, the effects of which may be biochemically similar to ammonium sulfate. It is not clear which specific chemical ingredient causes a crystallization effect using the aftershave. Creatine kinase catalyzes the conversion of creatine and consumes adenosine triphosphate (ATP) to create phosphocreatine and adenosine diphosphate (ADP). Crystallization hits occurred in 16 conditions, where six of these hits are over-the-counter pharmaceuticals and the remainder personal hygiene or cleaning products. We have no explanation for the effectiveness and grouping of these PSBs, having been unable to identify common chemical compounds. This is a clear case where X-ray structure determination could elucidate how the PSBs chemically influence crystal formation.

Lactoferrin (P) was an exciting case. Very significant increases in melting temperatures were seen for PSBs 52 (multivitamins, 28.2 K), 56 (prenatal vitamins, 23.7 K) and 58 (women's vitamins, 30.2 K). Lactoferrin is a multifunctional protein. Its roles include binding and transporting iron ions, and it also has antibacterial, antiviral, antiparasitic, catalytic, anticancer, antiallergic and radioprotection functions and

properties (Adlerova *et al.*, 2008). Both multivitamins and prenatal vitamins contained iron. While the ingredients for the women's vitamins were not available, it is likely that iron was present. An 11.2 K increase in melting temperature was seen for PSB 79, Fizzy Tub Colors. A number of compounds were present in this product. Crystallization hits occurred in PSBs 12 (Listerine fluoride rinse), 74 (Sugarfree Redbull) and 75 (Monster Energy drink), which all contain ingredients that would make them acidic. Redbull and Monster Energy drink have many chemical compounds in common. In the case of Listerine, the dyes used to color the product are included in the ingredient list. These coloring agents are present, but not identified, in the other two PSBs. It is not clear if the dyes are causing a protein-specific crystallization effect or if the effect is generic and due to a pH change caused by the PSB. Given the limited number of hits, and the fact that other acidic compounds do not have a similar effect, a case can be made for the investigation of the compounds used to provide artificial colors, as there is some evidence to support protein-specific interactions.

Concanavalin A (Q) saw significant increases in melting temperature (>10 K) for 11 of the 96 PSBs. These ranged from acidic conditions, *e.g.* Diet Coke (11.3 K), Monster Energy drink (11.3 K) and lemon juice (13.1 K), to basic conditions, *e.g.* baking soda (11.2 K). The PSBs that significantly altered the melting temperature also included mouthwashes, with Scope (6) producing the greatest change in melting temperature (28 K), as well as pepper (12.1 K) and maple extract (15.0 K). Concanavalin A is a carbohydrate-binding protein and undergoes a conformational change over the range pH 6–9, where the positively charged protein approaches a zero net charge (Zand *et al.*, 1971). The protein is promiscuous with respect to crystallization, with hits appearing in almost every cocktail with the notable exceptions of gel dishwashing detergent (28), Halls Defense supplement drops (57), raspberry coffee syrup (87) and hand soap (93). Interestingly, there was a decrease in the melting temperature of the protein (-4.6 K) for the raspberry coffee syrup, despite that PSB not producing a crystal hit.

Alcohol dehydrogenase (R) showed significant increases in melting temperature (>10 K) for five PSBs, 4 (grape juice, 10.4 K), 41 (aftershave, 13.5 K), 46 (cinnamon, 15.3 K), 47 (salt, 12.1 K) and 51 (prenatal vitamins, 10.4 K). Alcohol dehydrogenase catalyzes the conversion of primary unbranched alcohols to their corresponding aldehydes. We see no clear chemical correlation between the ingredients in these products, but note that many crystal hits are clustered in the 25% PSB solution, buffered at pH 5.8.

Catalase (S) shows a single increase in melting temperature >10 K for PSB 79, the Fizzy Tub Colors. Smaller increases in melting temperature are seen for Sudafed, Diet Coke, dill juice, dishwashing detergent and honey but, as for protein R, no common chemistry is readily identified. Catalase crystallizes in every PSB except vinegar (15).

Urease (S), an enzyme that catalyzes urea into CO_2 and NH_4 , is similar to concanavalin A in that there are pH-driven structural states. Two PSBs stand out with urease, 44 (pepper,

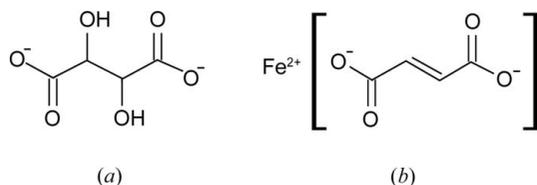


Figure 10

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.

5.8 K) and 60 (spices, 8.5 K). Interestingly, urease is thought to be inhibited by spices (Al Mofleh, 2010). Urease crystallizes in every cocktail except dishwashing liquid (31) and Softsoap hand soap (93).

While we observe useful information in the DSF results and crystallization screening, in the vast majority of cases an immediate correlation between the two sets of data is not readily apparent.

4. Discussion

We have presented a very limited glimpse into the data resulting from the first trials of this educational experiment. The teaching potential and analysis of the data are limited only by the educators' imaginations. In this case we taught students the scientific method by immersing them within it. While we provided the basic experimental skeleton and the protein samples of interest, the students decided which of the PSBs to incorporate into the experiment. In doing so they discovered that some initial choices were not suitable, because of solubility problems. We deliberately allowed the students to discover experimental problems, both during the formulation and later during the analysis. For a number of our compounds no information on the ingredients was available. When a hit occurred the students discovered that, without this initial information, the information that was derived from this somewhat blind hit was limited. An important aspect of these experiments and the students' education was the careful implementation and use of different types of controls. They discovered that good controls were imperative to ascertain whether the results were valid. Without adequate controls, the students learned that there was no way to determine if the result was potentially interesting or something else altogether. Unfortunately, we were unable to carry out a more detailed analysis as the majority of the students' time and effort were devoted to working out the experimental protocols. Most importantly, the students experienced the scientific process that is required to present valid results and defend those results to their peers and the scientific staff of HWI at the end of their internship. In this case they took this one step further, producing material that has given them insight into the communication of scientific knowledge through publication of their research.

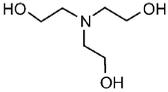
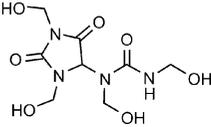
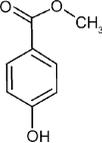
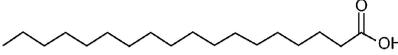
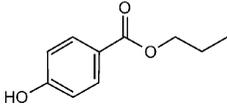
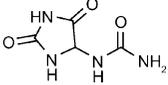
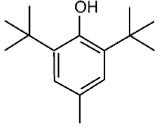
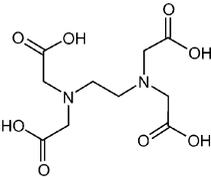
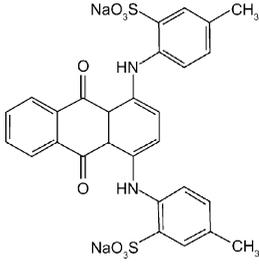
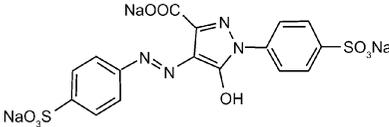
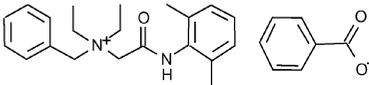
Our initial hypothesis was that the PSBs would affect proteins, either stabilizing or destabilizing them, and could be used as successful agents for crystallization. We saw that the former was true in the DSF experiments. However, our implicit assumption that some of these grocery store products could act in a similar manner to the commercially available Silver Bullets (Larson *et al.*, 2007a; McPherson & Cudney, 2006; McPherson *et al.*, 2007) was not rigorously tested with crystallographic analysis of the structures. While we certainly observed crystallization hits from the PSB experiments, we do not know if this was through specific small-molecule interactions with the protein, as associated with true silver bullets, or a more general chemical effect caused by ingredients that altered the solubility of the protein in a less specific manner,

e.g. by increasing the salt concentration or altering the pH. In Table 4 we show an example of the chemicals associated with one PSB chosen at random, aftershave lotion, number 41. We have not classified these chemical components in a similar manner to the commercially available Silver Bullets, nor did we make a conscious attempt to target specific classes of additives, as was the case for the Silver Bullets study (McPherson & Cudney, 2006), but it is clear that a diverse range of chemistry is represented in the PSBs. We do not have an answer from this investigation to the question of whether we are seeing a silver bullet crystallization effect; in some cases that appears to be the case but in others the evidence is far from conclusive. This leads into a discussion of the shortcomings of these initial experiments, of the educational process and of where we envisage the future of this project. In subsequent projects we would incorporate a characterization step, *e.g.* measurements of pH as a function of PSB and determining the relative concentration of the final PSB solution by measuring the refractive index. We would extend the work beyond the concept of PSBs into formulating more traditional cocktail screens from the grocery store. We would analyze our crystal hits with a UV microscope (recently incorporated into the crystallization screening pipeline) to determine if the crystals were indeed biological crystals and not crystallization artefacts (inorganic or small-molecule crystals). For the more advanced students we would incorporate an X-ray analysis step. This would determine if the chemical components of the PSB were involved in a protein-specific interaction, performing like 'real' silver bullets, or if the effect of the PSB was as a nonspecific chemical effect.

We focused this study on a binary outcome, 'crystals' or 'other'. In the classic crystallization phase diagram there are multiple outcomes, including precipitate, phase separation and clear, as well as crystals. With careful sampling of chemical space these can be used to derive vectors for optimization (Nagel *et al.*, 2008; Snell, Nagel *et al.*, 2008). In this case we have a random sampling of chemical space, but the results extend well beyond a 'crystal or no crystal' analysis and allow an opportunity to explore chemistry in a significantly more detailed manner, for example the effect of highly basic (bleach) or acidic (vinegar) compounds on biological systems. Of note was the dramatic effect that some of the PSBs had on stabilizing or destabilizing our targets. For example, Fizzy Tub Colors (79) stabilized lipase (11.7 K), while coconut conditioner (90) destabilized it to a similar extent (−11.7 K). In the case of lactoferrin, multivitamin (52), prenatal vitamins (56) and women's vitamins (58) all resulted in a greater than 20 K rise in the melting temperature for the protein – extremely significant. More interestingly, vinegar destabilized it, and so did Proactive revitalizing toner (8), with a −21.2 K change in melting temperature. These are examples of results that can be used to start a discussion on the body's interaction with the world around us. Almost 90% of the PSBs examined resulted in significant changes to the melting temperature for one or more of the 11 targets where DSF data were available. These limited data demonstrate that everyday products interact with biological systems. Whether that interaction is positive or

Table 4

The name, chemical structure and probable use of the non-PEG ingredients in PSB 41, aftershave lotion.

| Name | Structure | Use if known |
|---|---|---|
| Triethanolamine |  | pH balancer |
| Carbomer | A product class consisting of high molecular weight polymers of acrylic acid | Thickening, dispersing, suspending and emulsifying agents |
| Diazolidinyl urea |  | Antimicrobial preservative |
| Methylparaben |  | Anti-fungal agent |
| Stearic acid |  | Possibly used to produce a pearlescent effect |
| Propylparaben |  | Preservative |
| Allantoin |  | Moisturizer |
| BHT (butylhydroxytoluene) |  | Antioxidant additive |
| Disodium EDTA (shown without the two sodium ions) |  | Improves stability towards air, chelating agent |
| D&C Green No. 5 |  | Coloring agent |
| FD&C Yellow No. 5 |  | Coloring agent |
| Denatonium benzoate† |  | 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.

negative is often unknown, but the fact that it occurs serves as a starting point for a number of teachable discussions. This could be further exploited by a more selective choice of targets and appropriate PSBs.

It is important to note that this study was carried out by two undergraduate students during a ten-week summer internship. They were assisted by a third student and had direct supervision and assistance on the use of instrumentation from laboratory staff. Supporting these efforts was software development to make consistent and expedite the analysis of crystallization screening and DSF data. Two questions arise immediately. Was this study scientifically worthwhile? Could this concept be developed into an educational program that does not require ten weeks to complete? Although further crystallographic analysis is required to answer the first question definitively, our preliminary analysis demonstrates the validity and utility of using commercial products as multi-component chemical additives, with the potential to identify protein-specific silver bullets for macromolecular crystallization that fall outside the typical range of chemicals used in the crystallization laboratory.

The students' investigations focused on low-cost commercially available proteins and some rather offbeat choices of chemical additives. This was important as it piques the students' (and investigators') interest in the project and gave the students ownership of these experiments. Subsequent investigations would use proteins that are of structural interest in our laboratory and have proven recalcitrant to crystallization. We would use much more discretion in our selection of grocery store products to identify those that are chemically sensible to include in crystallization trials. If successful, there would be plenty of the commercial product available, but no knowledge of the exact concentration or chemical responsible for successful crystallization of the protein. If the X-ray diffraction from the crystal were sufficient and the chemical ordered in the structure, we could conceivably identify a specific chemical component bound to the protein, but non-specific chemical effects and the complex chemical formulations of these products could make it difficult to discern the exact component responsible for successful crystallization. That said, similar approaches have been successfully employed using multi-chemical component solutions for fragment cocktail soaks of protein crystals; Bosch *et al.* (2006) reported a study where 304 different compounds were prepared in 31 cocktails and used to identify novel lead compounds for *Trypanosoma brucei* nucleoside 2-deoxyribosyltransferase (EC 2.4.2.6). Fragment-based screening has utility for both X-ray crystallography and NMR, and, with high-throughput crystallization and diffraction screening, this approach has proved valuable for the development of pharmaceuticals (Jhoti *et al.*, 2007). Formulation of multi-component chemical cocktails can be challenging, with requirements for chemical compatibility and solubility generally undertaken as a trial-and-error approach. Commercial products alleviate this problem as they are supplied in a chemically stable and formulated state. Strictly from the perspective of crystallization and structural identification,

McPherson and co-workers have demonstrated that proteins can and will select specific small molecules from a multi-component solution and that often these compounds can be structurally resolved (Larson, Day & McPherson, 2010; Larson, Day, Nguyen *et al.*, 2010; Larson *et al.*, 2009a,b, 2008, 2007b).

While the approach of using grocery store products to identify silver bullets could at first sound gimmicky, it has proved to be a reasonable approach with scientific merit. We recognize that extensive trial and error could be required to resolve the specific chemical and concentration required for successful crystallization, and this requires protein samples, which are generally in short supply. An easier approach is simply to use the more traditional Hampton Research Silver Bullets or the more recently developed Hampton Research Silver Bullets Bio. Both screens are formulated and designed specifically for macromolecular crystallization and are more open to quantitative analysis of the results. However, these grocery store products offer interesting insight into the biological effects of the foods we eat, over-the-counter pharmaceuticals we ingest and lotions we apply to our skin. There is an opportunity to discover interactions that had previously gone unnoticed.

To answer the second question we have posed, could this project be developed into an educational program that does not require ten weeks to complete? Yes it can, and we envisage three approaches. The first is an intern arrangement similar to that described here but with collaborations with similar intern programs elsewhere, *e.g.* those at synchrotron facilities. This would allow more advanced students to follow the crystallization process to subsequent stages of the crystallographic process, ultimately leading to a structure with the potential to see the compounds bound to the protein. The second is a classroom-based approach. Pupils would be introduced to the science and then assigned to the task of developing a group of PSBs. A trip to the grocery store or local retailers to collect PSBs, collectively by the class or individually by the students, is one approach. However, this does not have to involve a trip to the grocery store. Each student could be encouraged simply to collect PSBs from their own home, with ingredients recorded, to eliminate any financial burden on the students. There would be a minimal set of supplies associated with this, *i.e.* small tubes for the sample, disposable pipettes and a 96-deep-well plate with a seal. Similarly, there would be minimal safety requirements beyond safety glasses and gloves if the educator chose to allow the use of certain household cleaning products (*e.g.* bleach was used in our study). After collecting and making 96 PSB solutions in a 96-well plate, the class would send their PSB plate to our facility. To set up a 1536-condition crystallization screening plate takes approximately 10 min, while to image the plate takes 20 min. Thus, imaging does not introduce a major impact on our existing pipeline. While we run the process eight hours a day, five days a week, we have spare capacity on the imaging tables and could if necessary extend the hours of their use.

A webcam would permit the students to watch as their experiments are set up robotically. There would be ample

opportunity for the teachers to discuss the chemistry of the products chosen and the expected results with the students. Images showing the outcomes of the crystallization screening experiments would be distributed and made available to the students on a weekly basis for four weeks. After the classroom receives the final results they would conduct an analysis. We have developed professional software for this and could adapt it to a classroom format. This is suited to a situation where there may be few facilities but competent educators to take advantage of the wealth of teaching examples. In the final version, we envisage a similar format as part of a week-long science camp. In this case we are not so much looking for crystals but using the same facilities to produce chemical reactions between proteins and grocery store products. We are actively looking to accomplish this in an appropriate program run by the Buffalo Museum of Science.

While we have focused on making use of high-throughput robotic facilities, coupled with internet access to the results and observations, the typical high-school science classroom can also undertake such a project without recourse to these facilities – high-throughput in a low-throughput manner. Materials to set up crystallization experiments are readily available and can be found in most high-school laboratories. One improvisation is the use of a relatively inexpensive plastic Petri dish with a Vaseline seal to set up hanging-drop vapor-diffusion experiments over a common reservoir (Fig. 11). If even modest funding is available, students can set up crystallization experiments using more traditional protocols that would include 24-well crystallization plates, with the help of excellent online guides available from the manufacturers. A pipette or syringe capable of delivering microlitre-scale solu-

tion volumes is required. If graduated pipettes with disposable tips are not readily available, a single 10 μl -volume Hamilton syringe can be used to set up crystallization experiments, with thorough washing in distilled water in between dispensing different chemical solutions. Another dispensing option is inexpensive disposable 1–5 μl -capacity Drummond Wiretrols. Styrofoam coolers, holding gel packs at room temperature, can be used to dampen temperature fluctuations, acting as an inexpensive substitute for a temperature-controlled incubator (and offering the ability for teaching the use of control experiments outside the cooler). The final requirement is a microscope. While the preferred microscope would be binocular with a variable range of magnifications (10 \times to 40 \times), almost any low-power microscope can be adapted for service.

The other materials that are required are the protein, the cocktail and the additive solutions if used. Combinations of proteins and corresponding cocktails, known to crystallize in a relatively short time frame, are well suited for these types of educational programs. Hampton Research (as an example) offers a selection of proteins that are prepared specifically for crystallization experiments, with recipes for crystallization provided on their website. Worthington Biochemical Corporation (Lakewood, New Jersey, USA) offers a substantial collection of enzymes at reasonable cost. These enzymes are typically lyophilized. While this is not ideal for crystallization trials, as the protein will often contain additional salts and some proteins refuse to crystallize after undergoing lyophilization, we have used them successfully for crystallization in our own laboratory. Worthington has an exceptionally informative online enzyme product manual with technical information that includes extensive references (including many covering crystallization). The broad selection of enzymes can enable a more expansive biochemical experience for the students. Educators can design projects where the students will have to use the literature to investigate and then rationally select commercial products or available chemicals that are likely to bind to and stabilize a particular enzyme to affect its crystallization. Another option is forming a partnership, *e.g.* with a local university or research institute, to obtain samples of interest.

The scope of the investigation is readily adjusted to the educational level of the students and the time and funding available for the program. The project can be scaled back by using fewer proteins and additives, and/or the instructor can set up the experiments, with the students responsible for providing and formulating a grocery store product to use as an additive beforehand. In this case the students' role then becomes focused on observation and analysis. Significantly less time is required in the classroom using this approach; the project could be set up during a single laboratory period and periodically monitored thereafter by the students. Likewise, the experiments can be expanded to provide a long-running project that requires part-time periodic observations, where the analysis is made over the course of several weeks. The pseudo-silver bullet crystallization program is readily integrated into a high-school science curriculum at very modest cost, while retaining its full educational impact. It is flexible

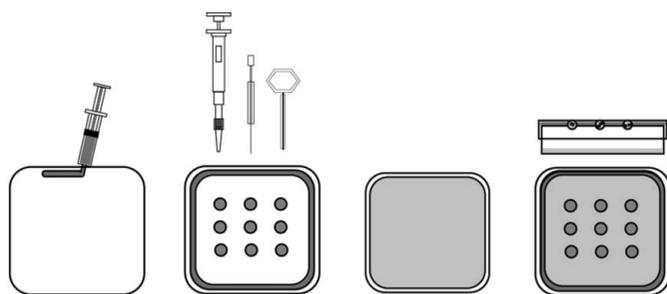


Figure 11

A simple-to-construct low-cost vapor-diffusion crystallization experiment using a plastic Petri dish. A disposable polystyrene Petri dish is easily adapted for setting up a hanging-drop vapor-diffusion crystallization experiment. The lid of the plate is removed and Vaseline applied around the inside rim using a syringe, or simply painting it on with a cotton swab. Once the grease seal is applied to the perimeter, the protein and crystallization cocktail drops are sequentially added to the inside of the lid (the same side as the grease seal). It is important to mark the lid so that drops can be identified later. Recommended volumes are 5 μl each of protein and cocktail, and 1.0 μl of any additive. Any of the illustrated devices (*e.g.* adjustable pipette, Hamilton syringe, Wiretrol) can be used to dispense the drops. The volumes of these components, the volume ratios, the order of adding the components, and mixing or leaving the drops undisturbed after delivery are all variables the students can pursue if a more comprehensive investigation is undertaken. A volume of reservoir solution is added to the bottom of the Petri dish (10 ml), and the lid is inverted over the bottom of the plate and pressed to ensure the Vaseline will form a good seal.

and offers the most talented and interested students opportunities for extra-curricular continuation of the project through an after-school program, a science club. The program can be expanded to challenge undergraduate or even graduate students who have the time and equipment to take on a more comprehensive study.

In our implementation we allowed the students to make predictions, taught them basic biochemistry and used the results to reinforce the outcomes. In order to know if we have been successful, assessment is an important part of the experience. As mentioned above, at the end of the study we have the students present their results in a meeting format to their peers and scientific staff of the institute. Students can work individually or in groups to demonstrate that they understand the experiments, using both human and library resources to prepare background and introductory material. The presentations prepared by the students not only prove that they understand the basic concepts of the experiment and that they carefully observed, concatenated and analyzed the results, but also teach them skills for successful presentation of material that carry well beyond scientific endeavors and will significantly benefit other aspects of their lives.

Going back to the first question, is this scientifically worthwhile? For the student examples we would include a couple of our favorite recalcitrant proteins that we can produce in large quantities. The program is certainly worthwhile as it might produce a crystal hit, but it is even more worthwhile as it will definitely involve students with the most primal component for scientific recruitment, the excitement of science – discovery. It teaches basic skills required for any scientific career: observation, accurate note-taking, solution handling, literature research and presentation. We see opportunities to include further concepts depending on the student level or background, *e.g.* the thermodynamics of crystallization, the effects of biophysical parameters or even protein properties that might have an influence on the process. To date, two groups of students have been involved with this program. As we develop it further we hope many more will gain from our efforts. From the enthusiasm of the students involved, we see it as scientifically worthwhile, not just from the immediate results but also from the application of the experience to future efforts.

5. Conclusion

We have used crystallization as a successful teaching tool. We have implemented a method where a school, college, university or other group could construct an array of pseudo-silver bullets and screen them against proteins without the need to purchase and maintain expensive facilities. We provide our results over the internet and the amount of data they contain allows detailed analysis covering a full range of academic levels. Where would we go for the next step? We have postulated that some of the PSBs may act in the same protein-specific manner as the real Silver Bullets (Larson *et al.*, 2007a; McPherson & Cudney, 2006; McPherson *et al.*, 2007). We have circumstantial evidence but no proof that this is the case.

Fortunately, we can go a step further in the analysis to resolve this question: once we have crystals we can obtain X-ray diffraction data from them. We have structures available for our targets, and visualizing the interaction between a protein and a PSB is a matter of producing a well diffracting crystal and using molecular replacement methods. With the use of high-throughput crystal optimization (Luft *et al.*, 2007), remote data-collection opportunities, *e.g.* our routine use of the SAM robot (Cohen *et al.*, 2002, 2005; Miller *et al.*, 2004) at SSRL, and the web-based data collection software *Blu-Ice* (Cohen *et al.*, 2005; McPhillips *et al.*, 2002) and *Web-Ice* (González *et al.*, 2008), we are poised for this to happen. We can escort a student from the aisle of their local grocery store to an experiment at a national facility where they can literally visualize the interaction of ingredients found in common household products with life on an atomic scale. They can be the first to discover a specific molecular interaction. This is in the future, but not that far off. One can imagine the teaching opportunities for a well coordinated science curriculum that integrates the diverse fields associated with crystallography into the visualization of life at its most basic level. The combination of high-throughput technologies, remote data collection, the internet and the humble grocery store make this entire process possible and readily achievable. Our next steps in this project will be to develop course materials for such a program and to recruit volunteers to test and further develop this unique opportunity for educational outreach for student interns and others in the years to come.

EHS and JRL conceived the experiment, helped implement it and analyze the data, and wrote the paper. NMF and REN created the PSB screen and performed most of the experiments and analysis, with oversight and assistance by JRW for formulation and crystallization, and by MES for DSF studies. JRW and MES helped in data analysis. EJP helped in image classification. SAP wrote the software to extract melting temperature from the DSF curves. We would like to acknowledge the US NIH (grant Nos. U54GM074899 and R01GM088396) and Dr George DeTitta at HWI for funding and allowing us to use the CHTSB High-Throughput Screening Laboratory for these experiments. The summer student intern program is run by Dr Jane Griffin and supported by Erie County, the East Hill Foundation, the Verizon Foundation, the Josephine Goodyear Foundation and the Ebenezer United Church of Christ, among others. We are very grateful for Dr Griffin's efforts and the generous supporters of the student intern program. We are grateful to the students who apply to the program. Finally, we would like to thank Wegmans, our local grocery store, who may not realize it but ended up making this research possible.

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