Supplementary Materials

Replacing a reservoir solution with desiccant in vapor diffusion protein crystallization screening

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Methods	Detailed description of the experimental protocols
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Figure S2	Weight evolution of silica gel at different temperatures
Figure S3	Heat treatment of the crystallization plates at 80°C
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Methods. Detailed description of the experimental protocols:

The conventional vapor diffusion method used in the reproducibility studies. The sitting drop method was used for reproducibility studies. (1) The reservoir solution was prepared by dissolving NaCl into NaAc buffer solution. (2) The crystallization solutions were prepared by dissolving protein into NaAc buffer solution and centrifuging (8,000 rpm for 30 s). The protein solution and the reservoir solution were then mixed at different volume ratios to make the crystallization solutions. (3) Reservoir solution (100 μl) was dispensed from the same stock solution into each of the reservoir wells using the automated crystallization robot. (4) The crystallization drops (2 μl) were dispensed from the same stock crystallization solution into each of the 96 sitting pits using the automated crystallization robot. The crystallization plates were then sealed using Crystal Clear Sealing Tape. (5) The plates were placed into a temperature-controlled chamber and incubated at a constant temperature (20ºC) for 5 days. (6) Images of the drops were captured and saved to a computer for subsequent analysis using an automated image reader.

The modified vapor diffusion method used in the reproducibility studies. A modified version of the sitting drop method was used in this study. (1) The silica gel was ground to generate small particles (~2 mm), exposed to the ambient environment, and allowed to absorb water for a sufficient amount of time (more than 4 weeks). A specified amount of particles was then distributed to each of the reservoir wells. This procedure was designed to ensure that the amount of silica gel distributed into each of the reservoir wells was the same. (2) The crystallization plates with distributed silica gel were placed in an oven and heated at 80ºC for 4 hours(see Supporting Information: Figure S6-7) to remove the absorbed water from the silica gel, and the plates were then immediately sealed using Crystal Clear Sealing Tape. (3) To make protein solutions, proteins were dissolved into NaAc buffer solutions and centrifuged (8,000 rpm for 30 s). The protein solutions and the reservoir solution were mixed at different volume ratios to make the crystallization solutions. (4) The crystallization solution (2 μl) was dispensed from the same stock solution into each of the 96 sitting pits, and the

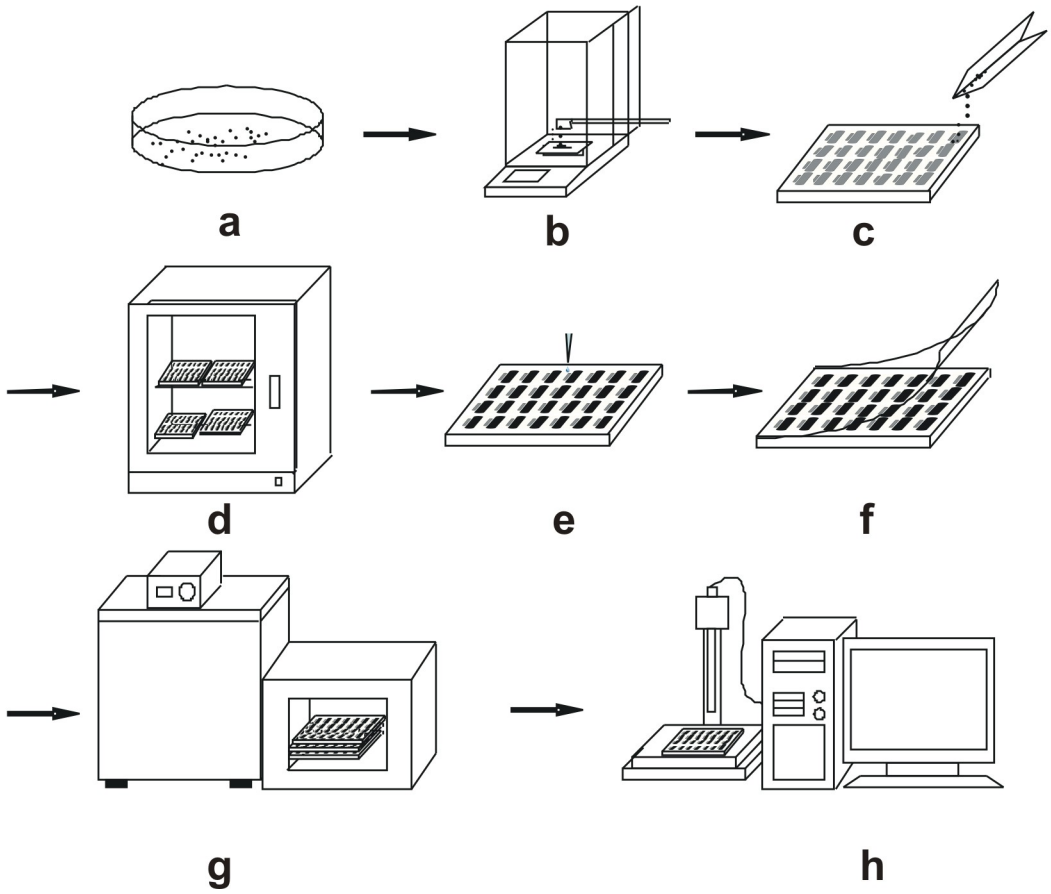
crystallization plates were sealed using Crystal Clear Sealing Tape. (5) The crystallization plates were placed into a temperature-controlled chamber and incubated at a constant temperature (20ºC) for 5 days. (6) Images of the drops were captured and saved to a computer for subsequent analysis using an automated image reader.

The conventional vapor diffusion method used in the screening test. The sitting drop method was used in the screening test. (1) The protein to be studied was dissolved in buffer solution and then centrifuged (8,000 rpm for 30 s) prior to use. (2) The reservoir solutions (100 μ l) were dispensed (i.e., the Index™ crystallization screening reagents) into the reservoir wells using the automated crystallization robot. (3) To set up the crystallization drops, 1 μ l of the reservoir solution was transferred from the reservoir wells to the corresponding sitting pits, and then 1 μ l of the protein solution was immediately dispensed into each of the sitting pits and mixed with the reservoir solution. The crystallization plates were sealed using Crystal Clear Sealing Tape. (4) The prepared crystallization plates were placed into a temperature-controlled chamber and incubated at a constant temperature (20ºC) for 5 days. (5) Images of the drops were captured and saved to a computer for subsequent analysis using an automated image reader.

The modified vapor diffusion method used in the screening test. A modified sitting drop method was used in the screening test. (1) The silica gel was ground to generate small particles (~2 mm), exposed to the ambient environment, and allowed to absorb water for a sufficient amount of time (more than 4 weeks). A specified amount of the particles was then distributed to each of the reservoir wells. This procedure was designed to ensure that the amount of silica gel distributed into each of the reservoir wells was the same. (2) The crystallization plates with the distributed silica gel were placed into an oven and heated at 80ºC for 4 hours to remove the absorbed water from the silica gel. The plates were sealed using Crystal Clear Sealing Tape. (3) To prepare the protein solution, protein was dissolved into buffer solution and then centrifuged (8,000 rpm for 30 s) prior to use. (4) The crystallization screening reagents (1 μ l;

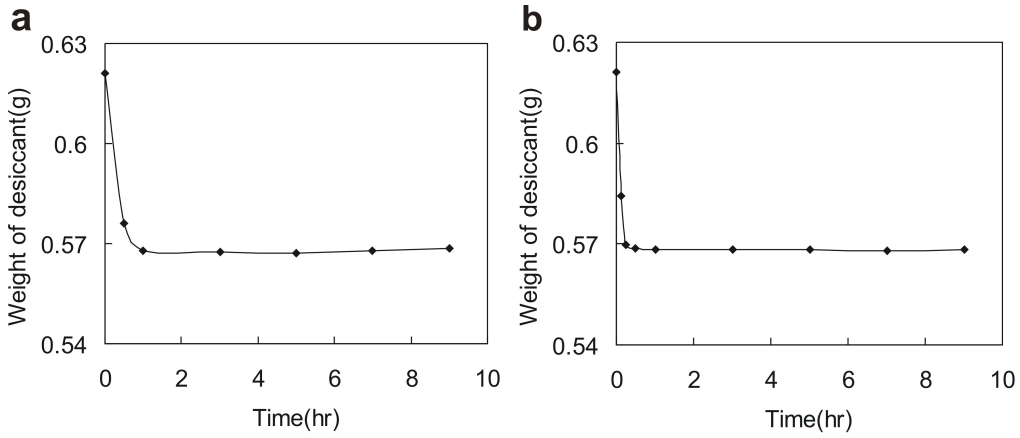
Index™) were transferred from a deep well plate to the sitting pits on the crystallization plates containing the distributed silica gel, and then 1 μl of the protein solution was dispensed into each of the sitting pits and mixed with the transferred reagents using the automated crystallization robot. The crystallization plates were sealed using Crystal Clear Sealing Tape. (5) The prepared crystallization plates were placed into a temperature-controlled chamber and incubated at a constant temperature (20ºC) for 5 days. (6) Images of the drops were captured and saved to a computer for subsequent analysis using an automated image reader.

Figure S1. Schematic illustration of the modified vapor diffusion method.



(a) Silica gel was ground into small particles and exposed to the ambient environment for more than 4 weeks; (b) the weight of the desiccant was measured; (c) the desiccant was evenly distributed into the reservoir wells; (d) the desiccant was dried in an oven at 80°C for 4 hours to drive out the absorbed water; (e) the crystallization drops were dispensed to the sitting pits of the crystallization plates; (f) the crystallization plate was sealed; (g) the crystallization solutions were incubated in a temperature controller; (h) the crystallization results were examined.

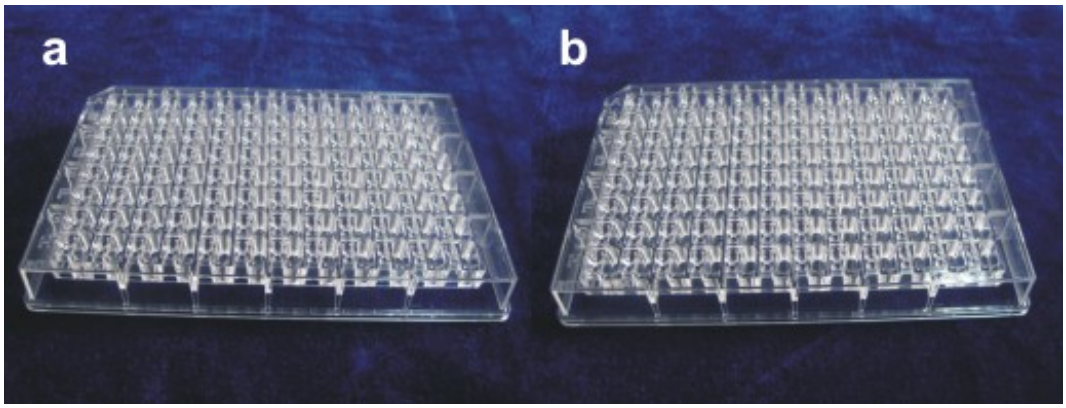
Figure S2. Weight evolution of silica gel at different temperatures.



We measured the weight evolution of the silica gel at 80ºC and 120ºC to determine the amount of time necessary to fully dry the silica gel.

The results show that 1 hour is long enough to fully dry the silica gel at 80ºC. In the current study, we dried the silica gel at 80ºC for 4 hours to ensure that the drying process was complete.

Figure S3. Heat treatment of the crystallization plates at 80°C.



The glass transition temperature of polystyrene is about 95°C. To determine whether the Intelli-plates (which are made of polystyrene) were resistant to treatment at 80°C, we incubated the Intelli-plates at 80°C for 8 h.
The results show that the Intelli-plates can withstand treatment at 80°C.

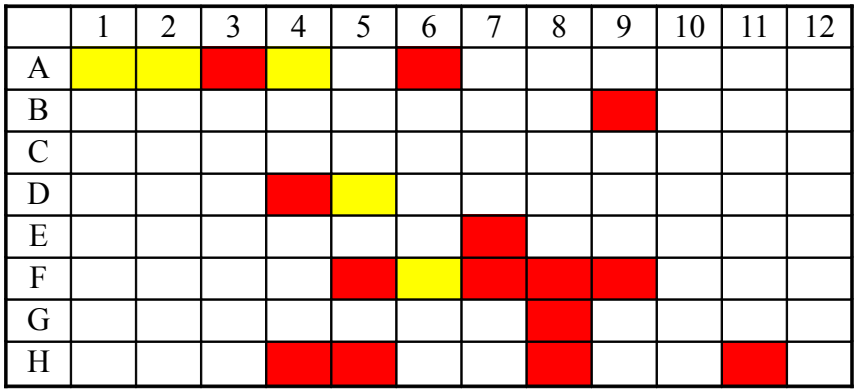
Figure S4. Comparisons of the crystallization screening hits obtained by the conventional and modified vapor diffusion methods.

hits in both the conventional and the modified vapor diffusion methods

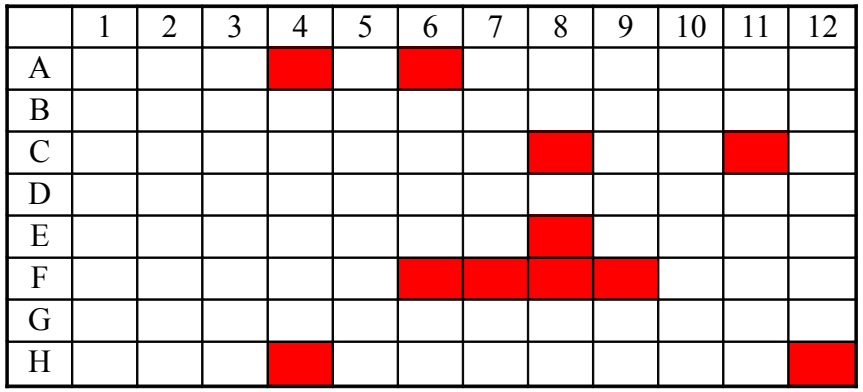
hits only in the conventional vapor diffusion method

hits only in the modified vapor diffusion method

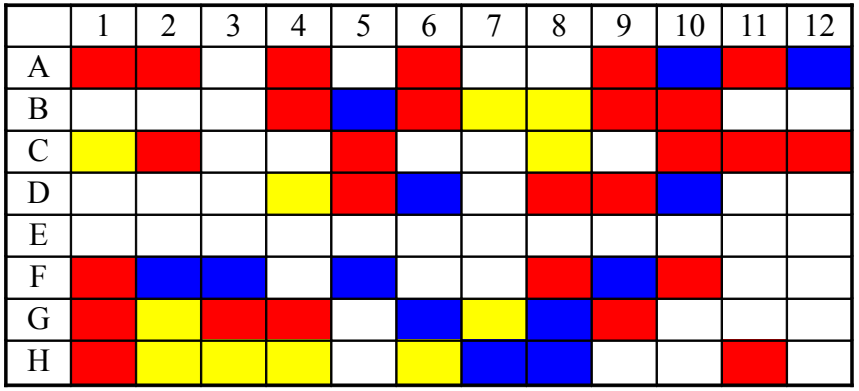
α-chymotrypinogen A II



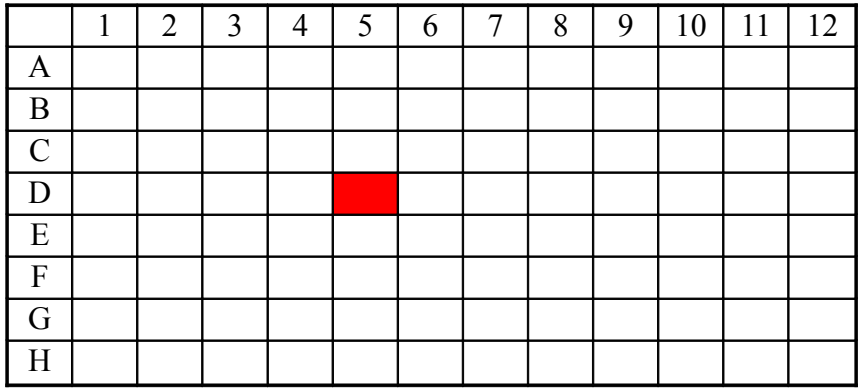
Ribonuclease A XII



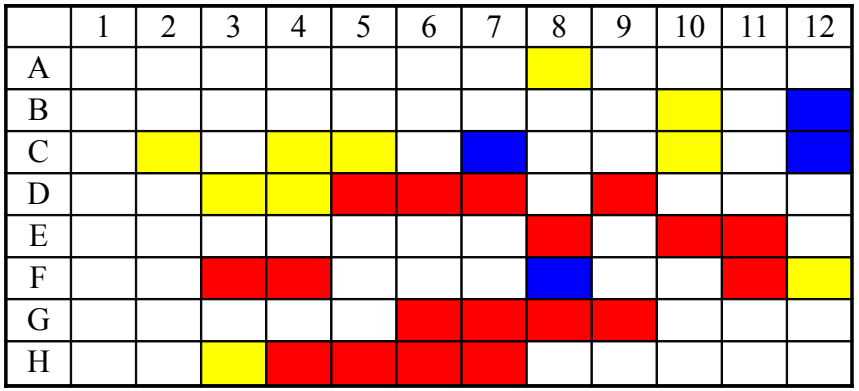
Proteinase K



Subtilisin A VIII



Lysozyme



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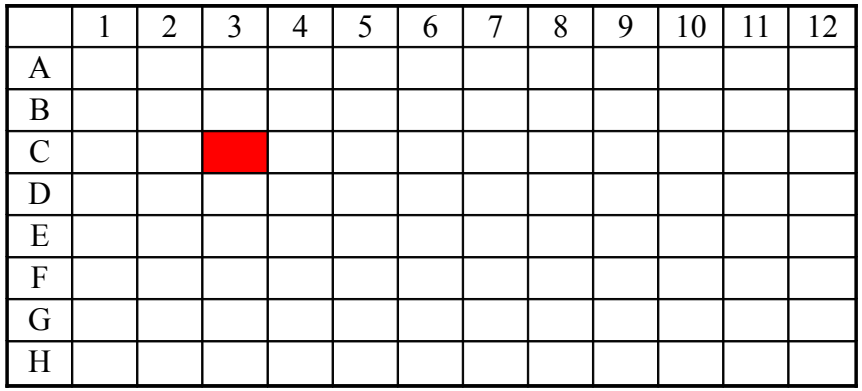
Myoglobin



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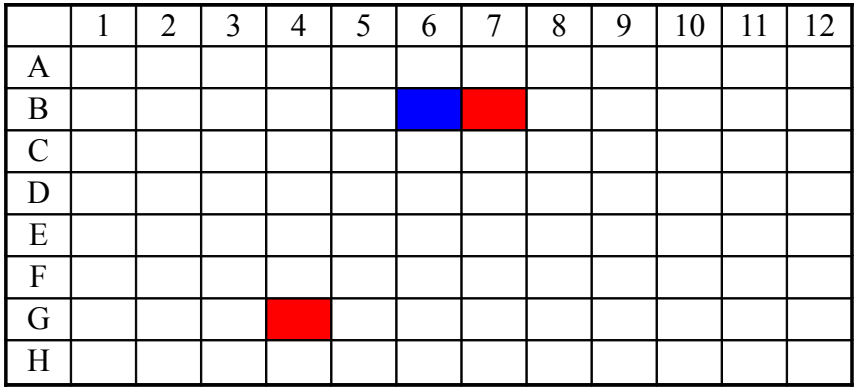
Cellulose



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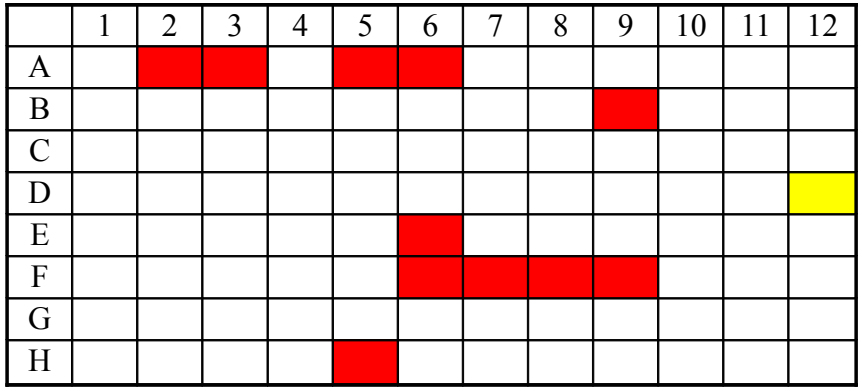
Ribonuclease A III



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Ribonuclease A I

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Thaumatin

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α-chymotrypinogen II

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Hemoglobin

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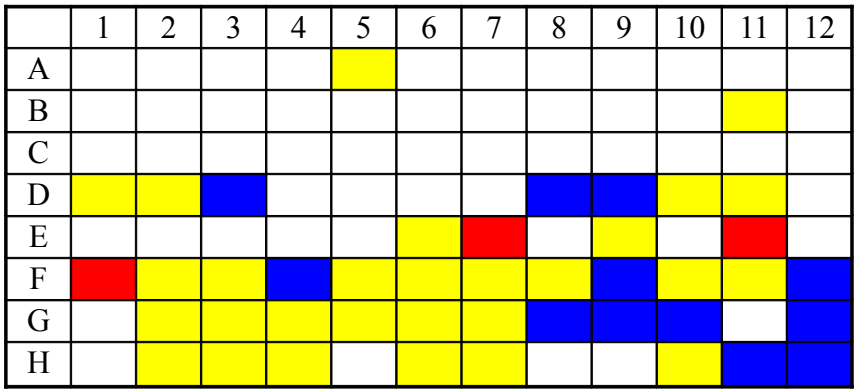
Concanavalin A VI



Note:

(1) Crystallization plates were 96-well sitting-drop Intelli-Plates (Hampton Research Co., HR3-143);

(2) Crystallization screening kits were Hampton Research Index™;

(3) Initial protein concentration (before mixing) was 10 mg/ml;

(4) The amount of silica gel in each reservoir well was 5 mg.

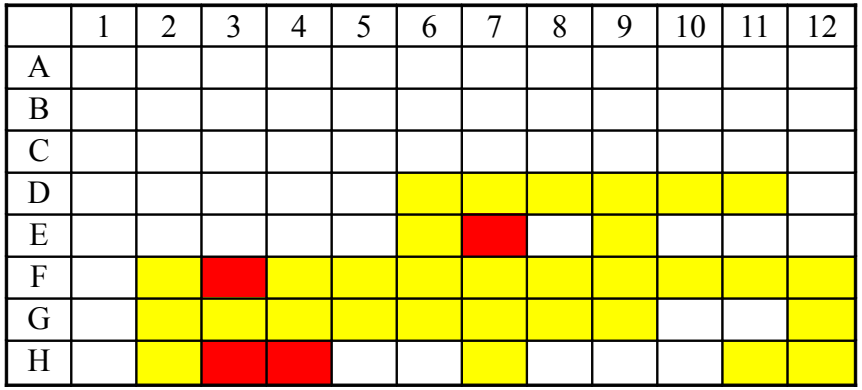
We analyzed the crystallization of 13 proteins using the conventional (sitting drop) vapor diffusion method and the modified (sitting drop) vapor diffusion method. For each of the tested proteins, all hits obtained from the 96 tested conditions are schematically shown in three colors. Yellow represents hits obtained with both methods; blue represents hits obtained only with the conventional method; red represents hits obtained only with the modified method.

The results show that the number of hits obtained only with the modified method (in red) was generally higher than the number of hits obtained only with the conventional method (in blue). The only exception was concanavalin A VI, which has extremely low solubility and may behave differently from the other proteins used.
It is noteworthy that five proteins (ribonuclease A I, ribonuclease A XII, subtilisin A VIII, myoglobin, and α-chymotrypsinogen II) did not yield crystals using the conventional method.

Figure S5. Comparisons of the crystallization screening hits obtained with the conventional and modified vapor diffusion methods at low initial protein concentrations (for proteins with very low solubility).

hits in both the conventional and the modified vapor diffusion methods
hits only in the conventional vapor diffusion method
hits only in the modified vapor diffusion method

Concanavalin A VI at initial concentration 2mg/ml (before mixing)



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Note:

(1) Crystallization plates were 96-well sitting-drop Intelli-Plates (HR3-143, Hampton Research Co.);

(2) Crystallization screening kits were Hampton Research Index™;

(3) Initial protein concentration (before mixing) was 2 mg/ml;

(4) The amount of silica gel in each reservoir well was 5 mg.

We tested crystallization of a protein with extremely low solubility (concanavalin A VI) at low concentrations. The results show that more hits were obtained with the modified method than with the conventional method

Table S1. Crystallization reproducibility studies using Linbro plates as compared with Intelli-Plates

Intelli-Plates (96-well plates, sitting drop)				Linbro-Plates (24-well plates, hanging drop)			
Conventional	Modified			Conventional		Modified	
Reservoir (μl)	Success rate (%)	Desiccant (mg)	Success rate (%)	Reservoir (μl)	Success rate (%)	Desiccant (mg)	Success rate (%)
100	0	5	20	600	0	1.0	50
						1.5	0
						2.0	0
						2.5	0
						3.0	0
						4.0	0
						5.0	0

We checked the effect of different volume of sealed space on the vapor diffusion crystallization by using Linbro boxes (24 well plates) as compared with that with the standard 96-well Intelli-Plates. The crystallization method is hanging-drop for the Linbro-plates, and sitting-drop for the Intelli-Plates. The crystallization conditions are as follows: the amounts of desiccant used were 1, 1.5, 2, 2.5, 3, 4, and 5 mg, and the drop volume was 2 microliters. The drop concentration conditions were No. 1 in Figure 2 (HEWL: 10 mg/ml. NaCl: 20 mg/ml; Reservoir solution: 40 mg/ml; Buffer: 0.1 M NaAc, pH 4.60). The temperature was 20℃. The incubation time was 2 days.

We found that only the crystallization experiment with 1 mg desiccant can yield a crystallization success rate of 50%. The droplets in the rest experiments were either dried-out or covered by a skin. These results showed that, the reservoir's area is important to the vapor diffusion process. The wells of the Linbro box possess larger volume than those of the 96-well plates, thus giving larger space for the drops to vaporize, reducing the amount of desiccant necessary. Furthermore, the skin appeared on the droplet indicated that the vapor diffusion may be too fast.