

Order from Chaos



The design and interpretation of high-throughput crystallization screens to guide optimization

Edward Snell

Acknowledgements



Ray Nagel for programming support

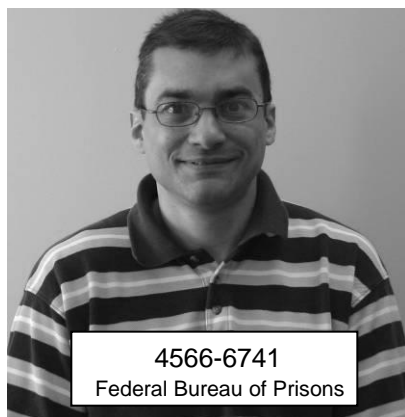
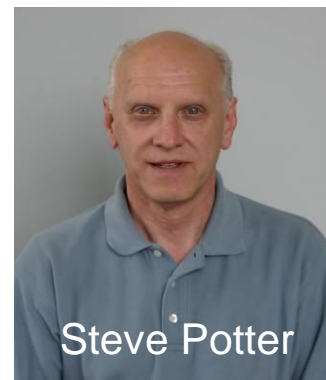
Joe Luft for discussions on the 1536 screen and analysis of the results



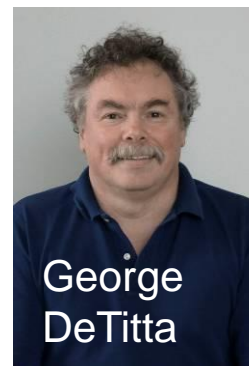
Melvin for the initial programming during his summer student project.



Ann, Miriem, Jen and Elizabeth for useful discussions, helping with the design of the chemical space, scoring images and testing the initial versions of the program.

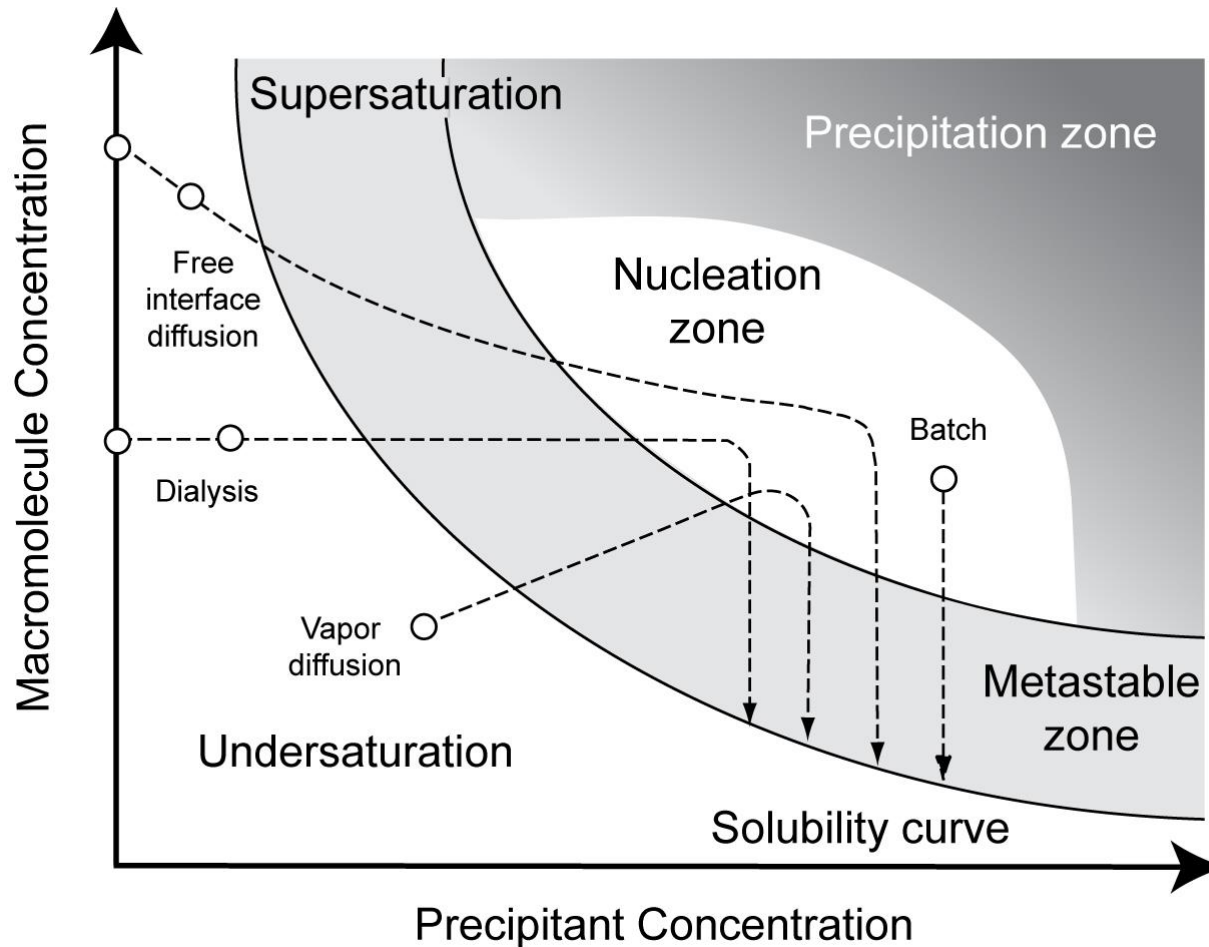


Steve for providing multiple macromolecule data for testing and George for supporting the research.

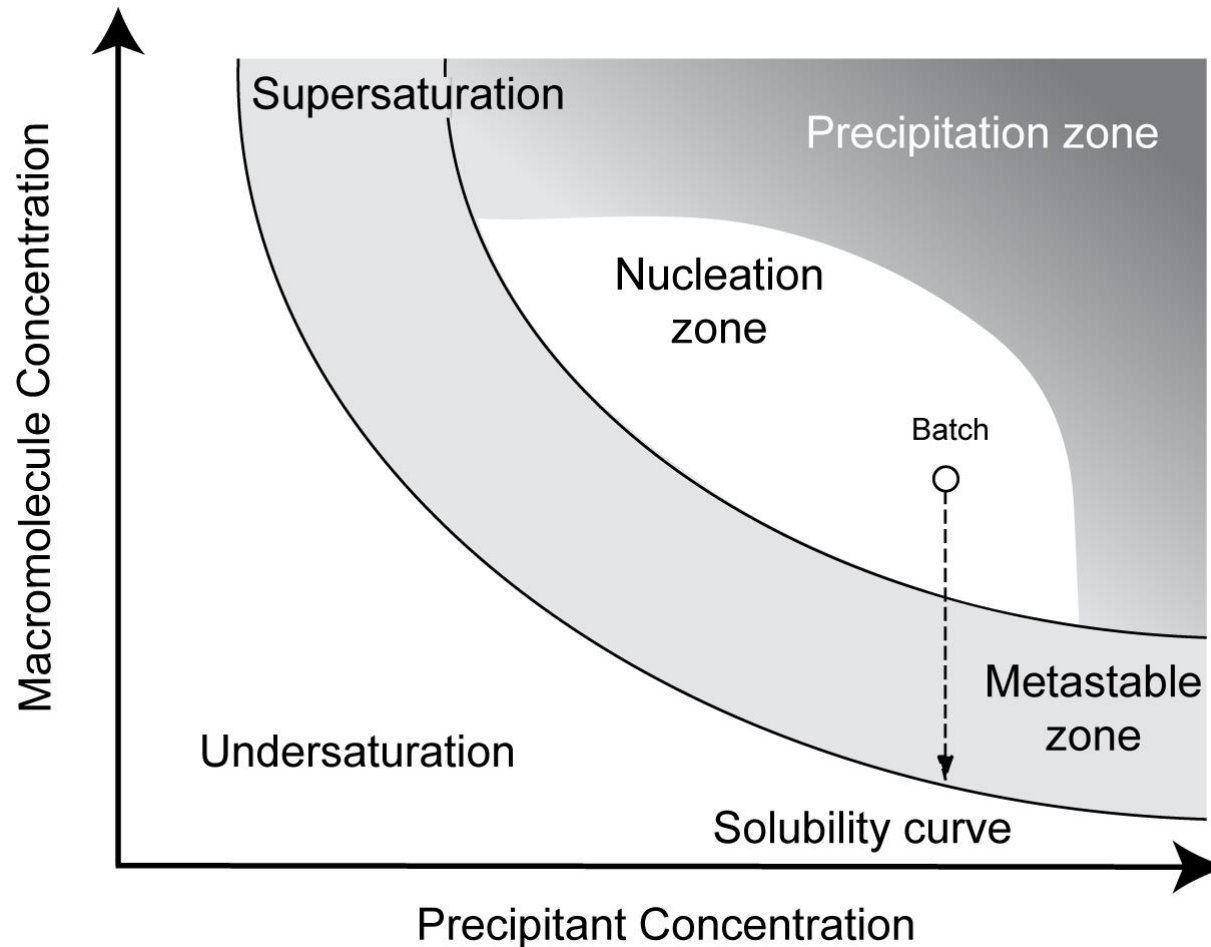


Not to forget the rest of the High-Throughput Lab, Tina, Angela and Ellie for putting up with me. Thanks also to Dean Myles, Hugh O'Neal and Flora Meilleur for samples.

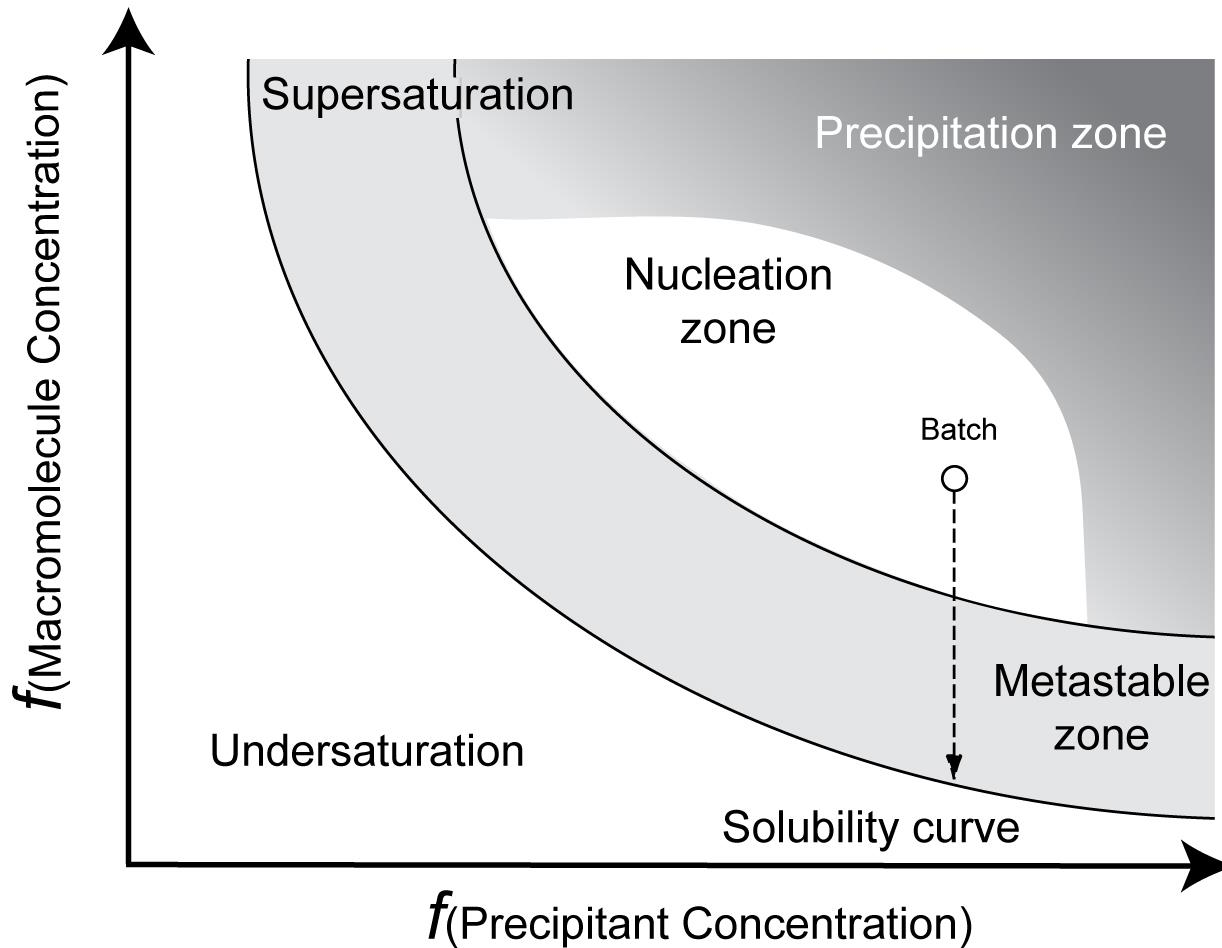
Simplified phase diagram for crystallization



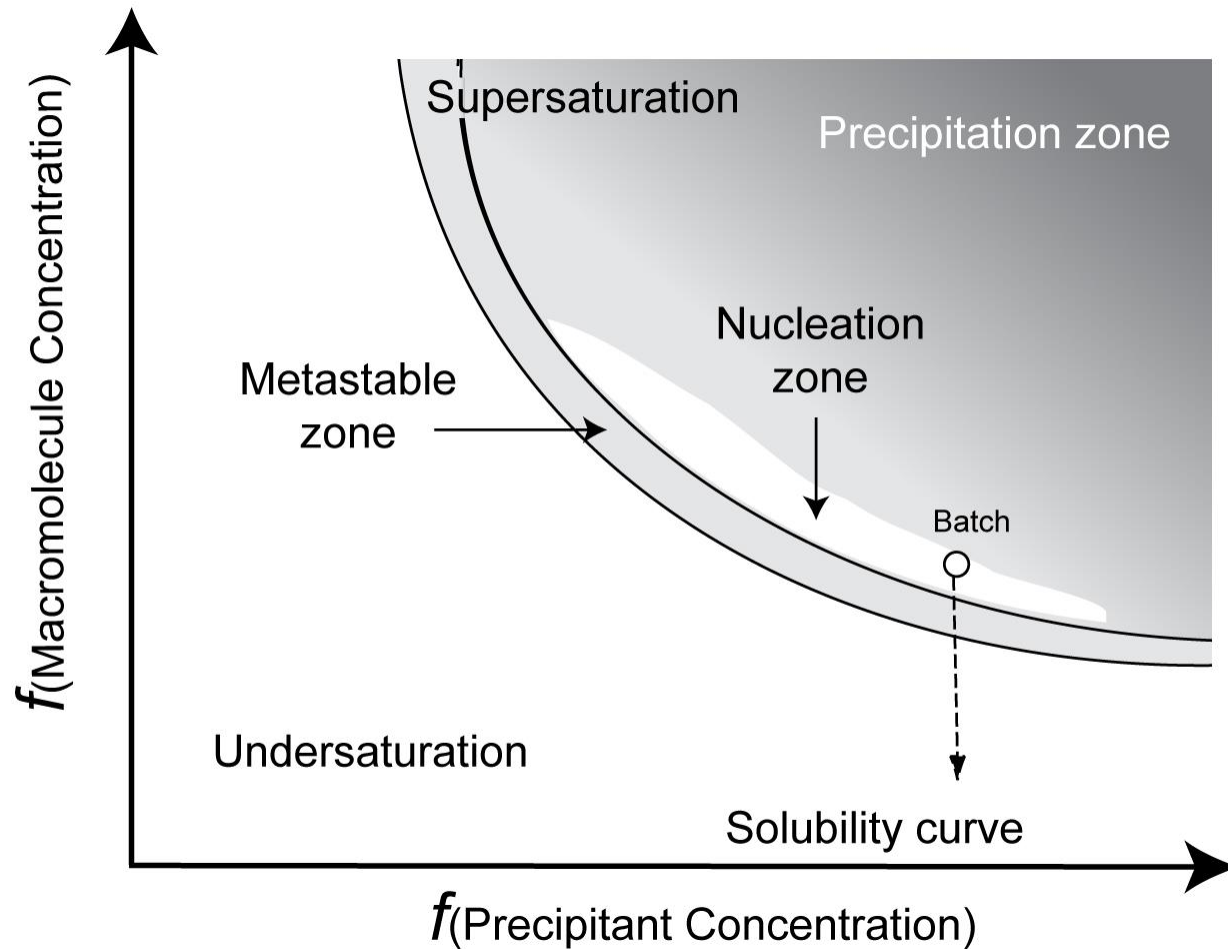
Even simpler phase diagram for crystallization



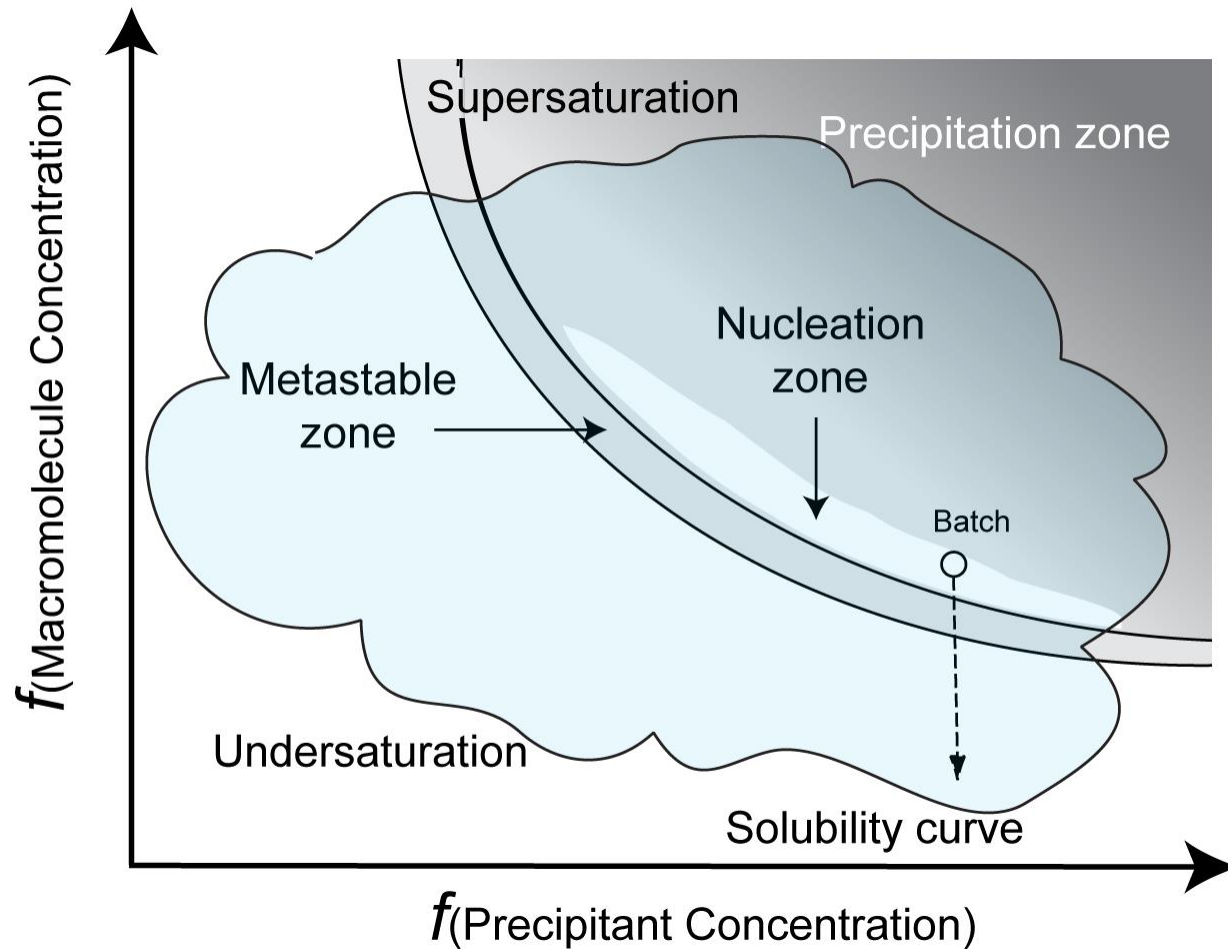
Start to throw some reality into the equation



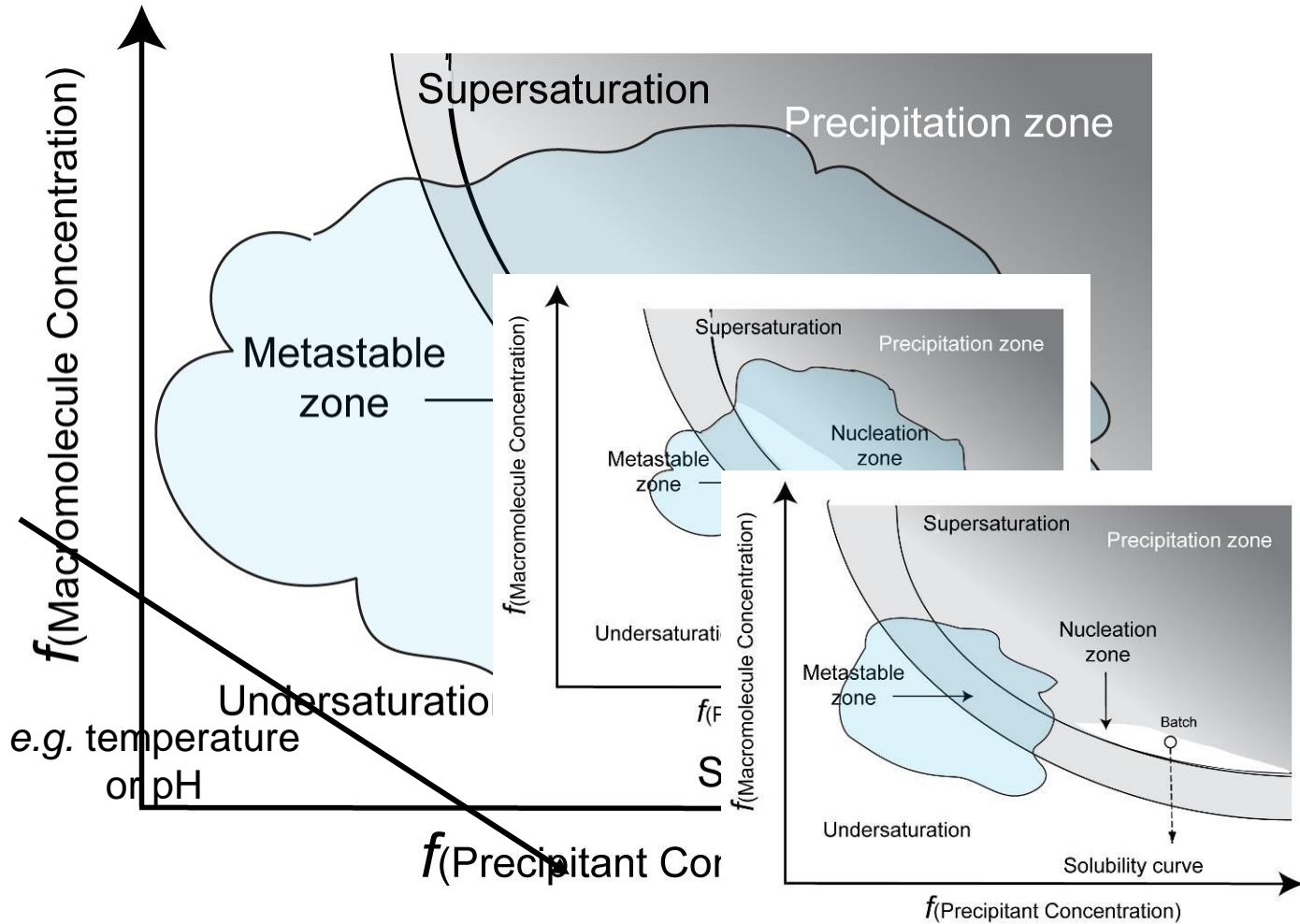
And reduce the chances of crystallization a little



Add the experimental space we sample



And the fact that it's not just two dimensions



Lets introduce a typical crystallographer ...

Wile E. Coyote (Genius)



Overconfidentii Vulgaris

(Cristali Coltivatore Optimista)

And the crystal of interest ...

Road Runner
(Beep beep)



Disapparialis Quickius

(Cristallio Perfetto)

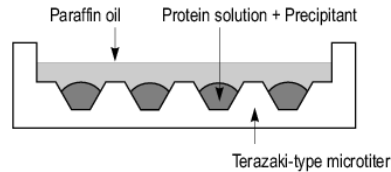
And how the rules of the crystallographer relate to crystallography ...

1. Road Runner cannot harm the Coyote except by going "Beep! Beep!"
2. No outside force can harm the Coyote - only his own ineptitude or the failure of Acme products.
3. The Coyote could stop anytime - If he was not a fanatic.
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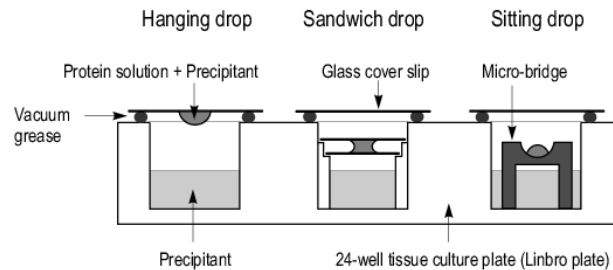
1. The crystal cannot harm the crystal grower except by not diffracting.
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Crystallizing Macromolecules

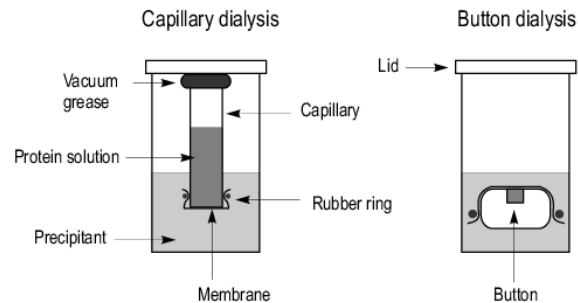
a) Microbatch crystallisation technique



b) Vapour-diffusion techniques



c) Dialysis crystallisation techniques



Many different methods but they all have things in common:

- They are designed to traverse the crystallization phase diagram.
- They use many different kinds of solutions to sample crystallization space at many points.

Catching Road Runners



Growing Crystals



Crystallization is complex



How do we grow crystals?

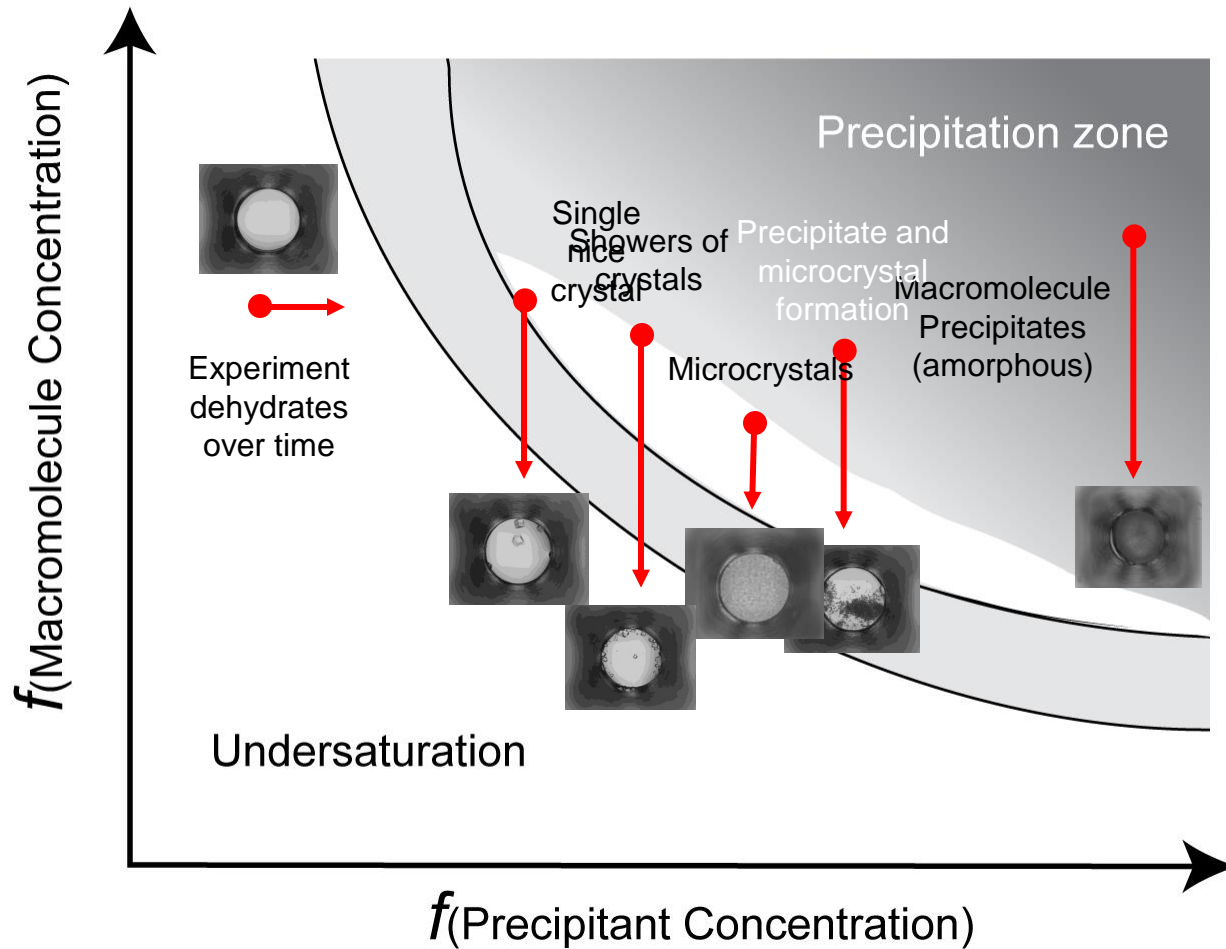
- Multiple guess?
- Intelligent design?

Set up many small scale experiments in conditions likely to be favorable for crystallization

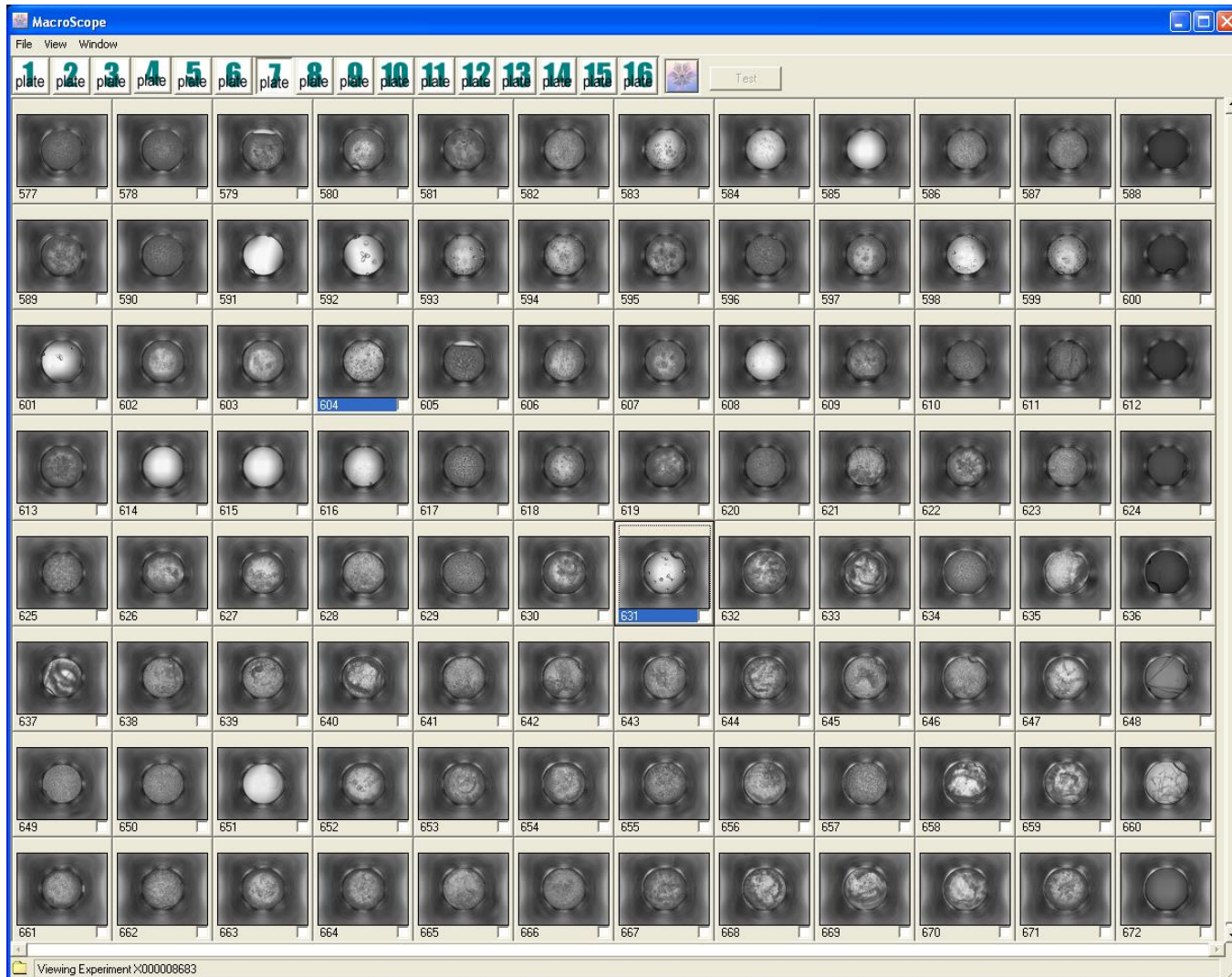
- Limited by amount of sample, time and effort.
- How many conditions is optimum? Divergent views (we'll return to this later)

Lets do the experiment

What results can we expect to see?



What do we actually see?

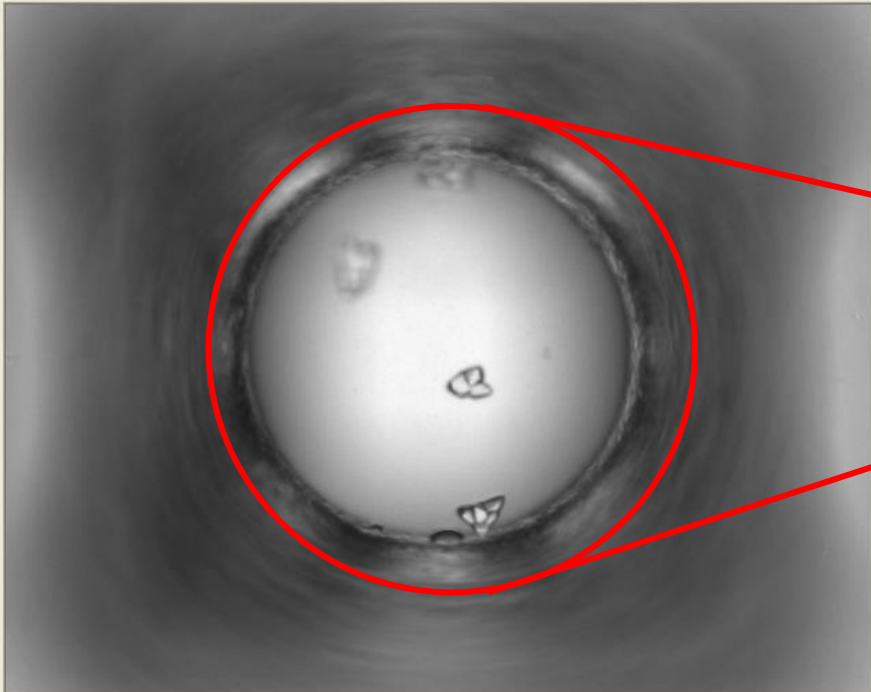


What do we actually see?

Full Image

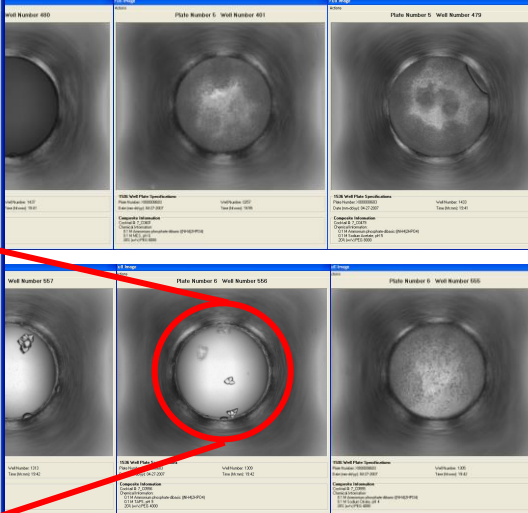
Actions

Plate Number 6 Well Number 556



1536 Well Plate Specifications
Plate Number: X000008683 Well Number: 1309
Date (mm-dd-yy): 04-27-2007 Time (hh:mm): 19:42

Composite Information
Cocktail #: 7_C0556
Chemical Information:
0.1 M Ammonium phosphate-dibasic ((NH₄)₂HPO₄)
0.1 M TAPS, pH 9
20% (w/v) PEG 4000



Well Number 550 Plate Number 6 Well Number 551 Plate Number 6 Well Number 552

Well Number 553 Plate Number 6 Well Number 554 Plate Number 6 Well Number 555

Well Number 556 Plate Number 6 Well Number 557

Well Number 558 Plate Number 6 Well Number 559

Optimize crystals by screening around the hit conditions, *i.e.* 0.1 M ammonium phosphate dibasic, 0.1 TAPS pH 9 and 20% (w/v) PEG 4000

Remember how the rules of the crystallographer relate to crystallography ...

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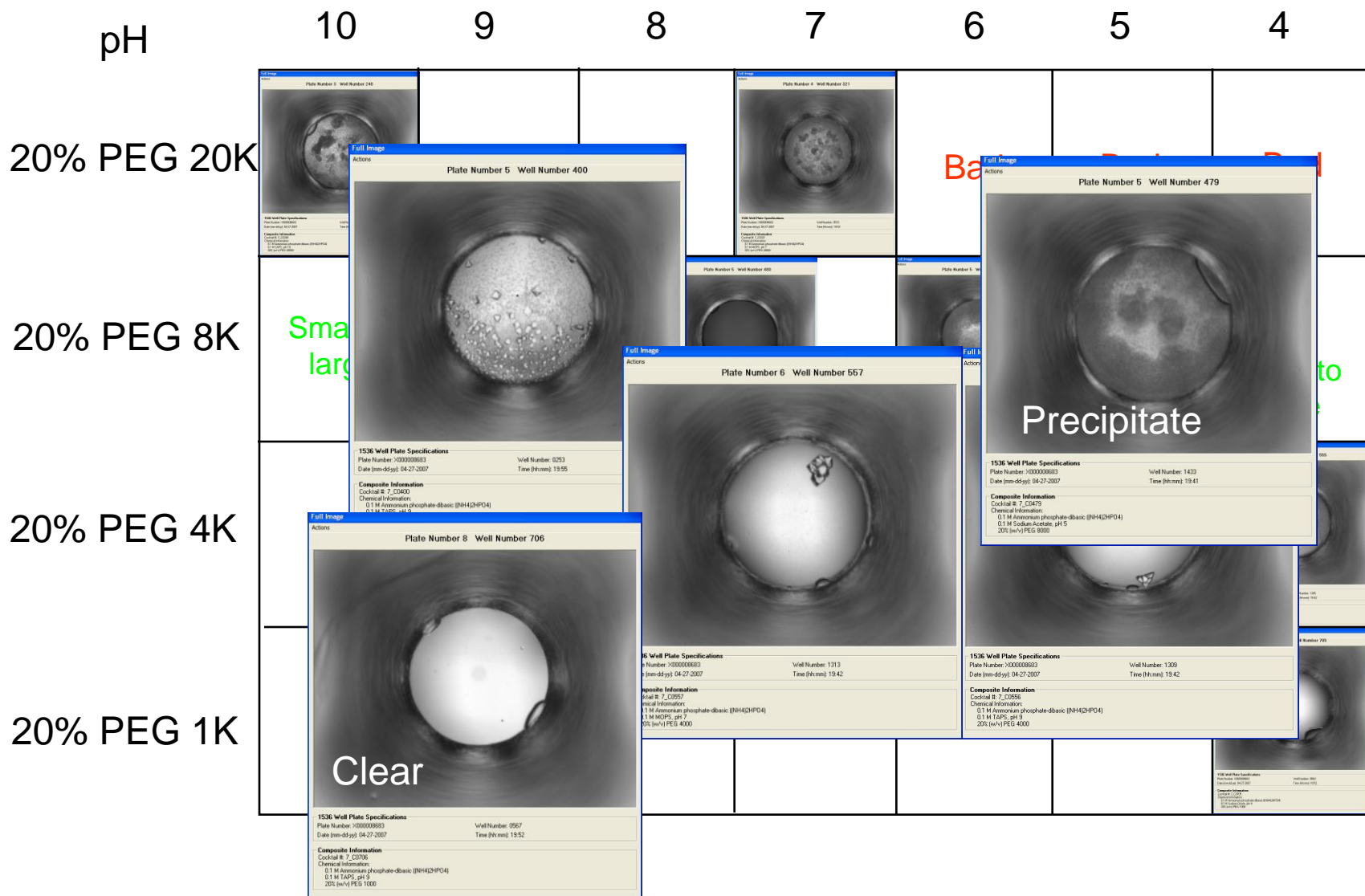
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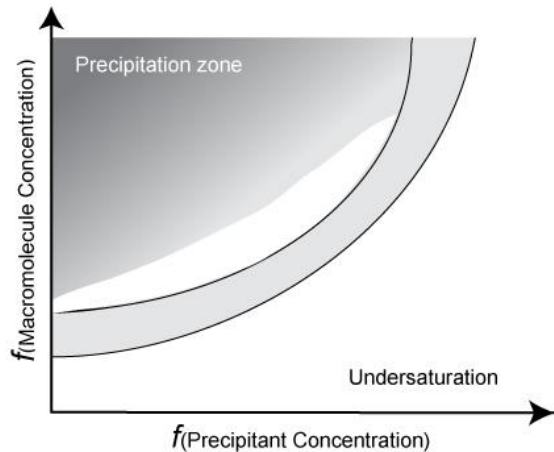
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If we plot the results in chemical space the road becomes clear

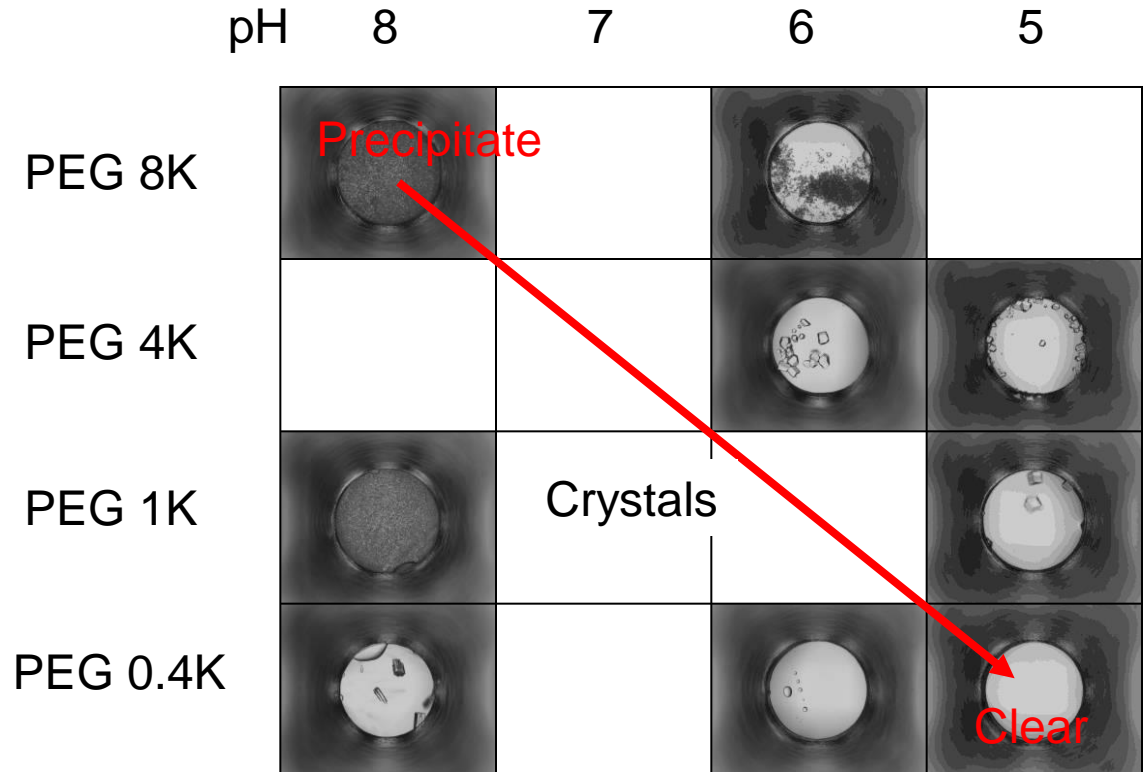


Chemical space provides a vector for optimization

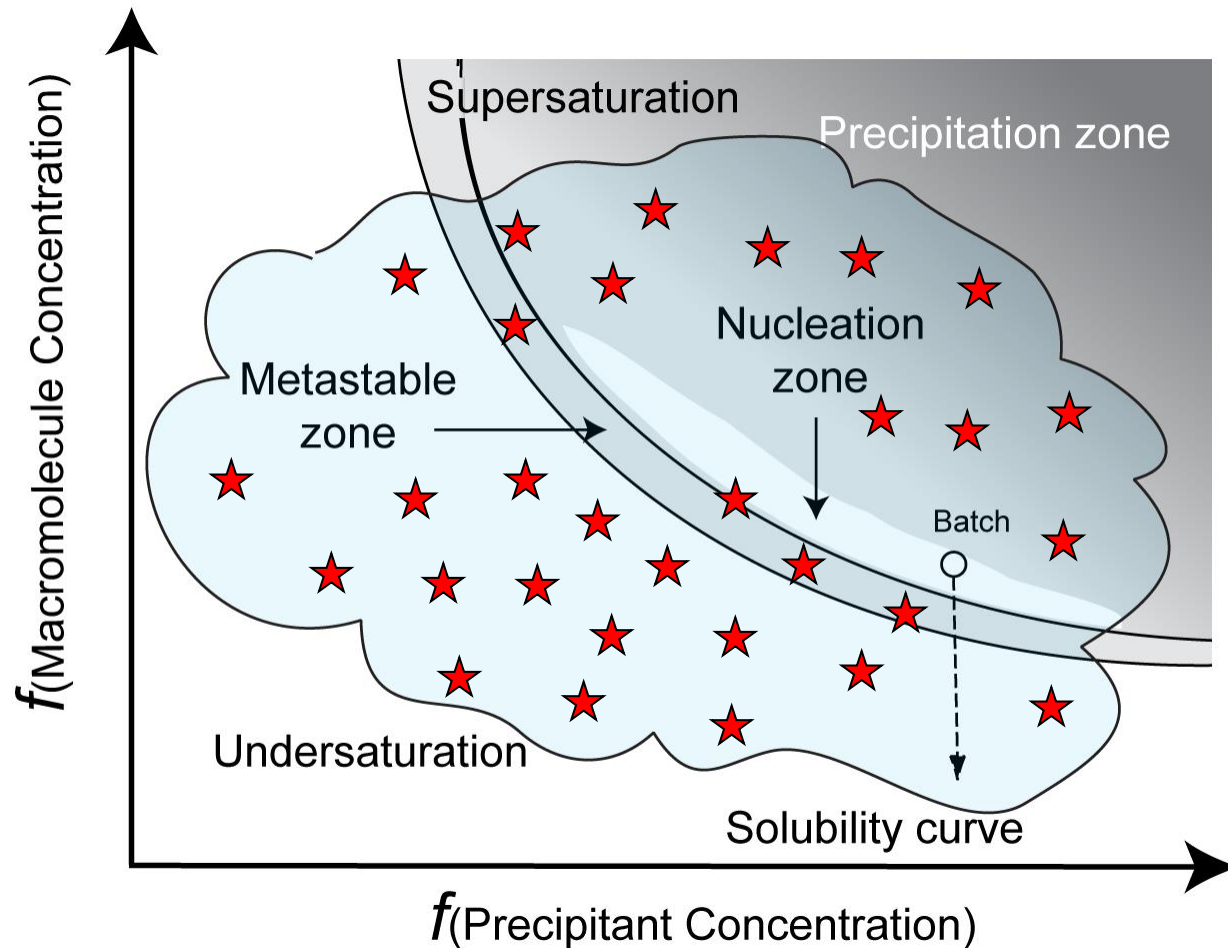
In this case the path from precipitate through crystals to clear is obvious. The phase diagram is reversed. Also clear are the number of chemical conditions that have not been sampled.



Ubiquitin, 40% PEG, 0.1M zinc acetate



It also illustrates the space we do not sample



We only sample discrete points within the sampling space

The HWI crystallization cocktail screen.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48		
Ammonium										Monobasic and dibasic phosphate										Sulfate										Calcium																			
Bromide										Chloride										Nitrate										Acetate										Chloride									
49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96		
Lithium										Magnesium										Manganese										Sulfate																			
Bromide										Chloride										Acetate										Nitrate																			
97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144		
Acetate										Carbonate										Chloride										Nitrate																			
Rubidium										Sulfate										Thioacetate										Thioacetate																			
Chloride										Bromide										Sulfate										Nitrate																			
193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237					
Zinc										Potassium										Lithium										Potassium																			
Acetate										Phosphate dibasic										Sulfate heptahydrate										Sulfate monohydrate																			
Highly soluble salt, cation and anion screen																																																	
↑																																																	
241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288		
PEG 20000 20% (v/v)										PEG 20000 20% (v/v)										PEG 20000 20% (v/v)										PEG 20000 20% (v/v)																			
Chloride										Nitrate										Sulfate										Phosphate																			
289	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336		
PEG 20000 40% (v/v)										PEG 20000 40% (v/v)										PEG 20000 40% (v/v)										PEG 20000 40% (v/v)																			
Sodium										Potassium										Lithium										Magnesium																			
Bromide										Chloride										Nitrate										Sulfate																			
337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384		
PEG 20000 40% (v/v)										PEG 20000 40% (v/v)										PEG 20000 40% (v/v)										PEG 20000 40% (v/v)																			
Potassium										Rubidium										Sodium										Zinc																			
Chloride										Nitrate										Sulfate										Phosphate																			
385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432		
Ammonium										Lithium										Magnesium										Potassium																			
Thioacetate										Sulfate										Nitrate										Phosphate																			
PEG 8000 Screen																																																	
←																																																	
433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480		
PEG 8000 20% (v/v)										PEG 8000 20% (v/v)										PEG 8000 20% (v/v)										PEG 8000 20% (v/v)																			
Sodium										Potassium										Lithium										Magnesium																			
Bromide										Chloride										Nitrate										Sulfate																			
481	482	483	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524	525	526	527	528		
PEG 8000 40% (v/v)										PEG 8000 40% (v/v)										PEG 8000 40% (v/v)										PEG 8000 40% (v/v)																			
Ammonium										Potassium										Lithium										Magnesium																			
Sulfate										Chloride										Nitrate										Sulfate																			
529	530	531	532	533	534	535	536	537	538	539	540	541	542	543	544	545	546	547	548	549	550	551	552	553	554	555	556	557	558	559	560	561	562	563	564	565	566	567	568	569	570	571	572	573	574	575	576		
PEG 8000 40% (v/v)										PEG 8000 40% (v/v)										PEG 8000 40% (v/v)										PEG 8000 40% (v/v)																			
Sodium										Potassium										Lithium										Magnesium																			
Bromide										Chloride										Nitrate										Sulfate																			
577	578	579	580	581	582	583	584	585	586	587	588	589	590	591	592	593	594	595	596	597	598	599	600	601	602	603	604	605	606	607	608	609	610	611	612	613	614	615	616	617	618	619	620	621	622	623	624		
PEG 4000 20% (v/v)										PEG 4000 20% (v/v)										PEG 4000 20% (v/v)										PEG 4000 20% (v/v)																			
Magnesium										Potassium										Lithium										Magnesium																			
Chloride										Nitrate										Sulfate										Phosphate																			
625	626	627	628	629	630	631	632	633	634	635	636	637	638	639	640	641	642	643	644	645	646	647	648	649	650	651	652	653	654	655	656	657	658	659	660	661	662	663	664	665	666	667	668	669	670	671	672		
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Ammonium										Potassium										Lithium										Magnesium																			
Sulfate										Chloride										Nitrate										Sulfate																			
673	674	675	676	677	678	679	680	681	682	683	684	685	686	687	688	689	690	691	692	693	694	695	696	697	698	699	700	701	702	703	704	705	706	707	708	709	710	711	712	713	714	715	716	717	718	719	720		
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Sodium										Potassium										Lithium										Magnesium																			
Bromide										Chloride										Nitrate										Sulfate																			
721	722	723	724	725	726	727	728	729	730	731	732	733	734	735	736	737	738	739	740	741	742	743	744	745	746	747	748	749	750	751	752	753	754	755	756	757	758	759	760	761	762	763	764	765	766	767	768		
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Ammonium										Potassium										Lithium										Magnesium																			
Sulfate										Chloride										Nitrate										Sulfate																			
769	770	771	772	773	774	775	776	777	778	779	780	781	782	783	784	785	786	787	788	789	790	791	792	793	794	795	796	797	798	799	800	801	802	803	804	805	806	807	808	809	810	811	812	813	814	815	816		
PEG 1000 40% (v/v)										PEG 1000 40% (v/v)										PEG 1000 40% (v/v)										PEG 1000 40% (v/v)																			
Ammonium										Potassium										Lithium										Magnesium																			
Sulfate										Chloride										Nitrate										Sulfate																			
817	818	819	820	821	822	823	824	825	826	827	828	829	830	831	832	833	834	835	836	837	838	839	840	841	842	843	844	845	846	847	848	849	850	851	852	853	854	855	856	857	858	859	860	861	862	863	864		
PEG 1000 40% (v/v)										PEG 1000 40% (v/v)										PEG 1000 40% (v/v)										PEG 1000 40% (v/v)																			
Sodium										Potassium										Lithium										Magnesium																			
Bromide										Chloride										Nitrate										Sulfate																			
865	866	867	868	869	870	871	872	873	874	875	876	877	878	879	880	881	882	883	884	885	886	887	888	889	890	891	892	893	894	895	896	897	898	899	900	901	902	903	904	905	906	907	908	909	910	911	912		
PEG 400 Screen										PEG 400 Screen										PEG 400 Screen										PEG 400 Screen																			
←																																																	
PEG 400 20% (v/v)										PEG 400 20% (v/v)										PEG 400 20% (v/v)										PEG 400 20% (v/v)																			
Sodium										Potassium										Lithium										Magnesium																			
Bromide										Chloride										Nitrate										Sulfate																			
913	914	915	916	917	918	919	920	921	922	923	924	925	926	927	928	929	930	931	932	933	934	935	936	937	938	939	940	941	942	943	944	945	946	947	948	949	950	951	952	953	954	955	956	957	958	959	960		
PEG 400 80% (v/v) 80%										PEG 400 20% (v/v)										PEG 400 80% (v/v)										PEG 400 80% (v/v) 80%																			
Ammonium										Potassium										Lithium										Magnesium																			
Sulfate										Chloride										Nitrate										Sulfate																			
961	962	963	964	965	966	967	968	969	970	971	972	973	974	975	976	977	978	979	980	981	982	983	984	985	986	987	988	989	990	991	992	993	994	995	996	997	998	999	1000	1001	1002	1003	1004	1005	1006	1007	1008		
PEG 400 80% (v/v) 80%										PEG 400 20% (v/v)										PEG 400 80% (v/v)										PEG 400 80% (v/v) 80%																			
Sodium										Potassium										Lithium										Magnesium																			
Bromide										Chloride										Nitrate										Sulfate																			

The 1536 diverse chemical cocktails (Luft et al., 2003). The 984 in-house conditions comprise an incomplete factorial sampling of 36 salts, eight buffers, and 5 different PEGs.

The remainder of 1536 cocktails are comprised of commercial salts available from Hampton Research. Specifically, in order of use; the Matrix Screen, Quick Screen, Nucleic Acid Screen, Sodium Malonate Grid, PEG/Ion, PEG 6000 Grid, Ammonium Sulfate Grid, Sodium Chloride Grid, HT Screen, Index and the SaltRx screen.

A special case – The Hampton Research Index Screen

Hampton Research Index Screen																			
Note, the HT screen is not a conventional screen as such. It is designed to sample a range of reagents and provide an indication of the appropriate chemical area and variables that would be appropriate for crystallization and should be used in this manner.																			
pH	Ammonium Sulfate 2.0M		Sodium chloride 3.0M		Magnesium formate dihydrate		Sodium phosphate		Neutralized organic acids (pH 7.0)		High supersaturation salt and low polymer		Low ionic strength systems		Non-volatile organics				
	pH				0.3M	0.5M			pH										
3.5	A1	A7						5.6	B5			5.5	C8		3.5	D4	D12		
4.5	A2	A8						6.9	B6			6.5	C6		4.5	D5	E2		
5.5	A3	A9			B1			8.2	B7			8.5	C7		5.5	D6	E1		
6.5	A3	A10				B2							C9			D7	E3		
7.5	A5	A11			B3								C10		6.5	D10	E6		
8.5	A6	A12				B4						7	C11			D11	E9		
													C12				E10		
	Classic salt versus pH														7		D2		
															7.5		D3		
															8.5		D8		
	Hits here indicate that a variation of salt concentration and pH in a grid screen has a strong potential for crystallization																D9		
																		E4	
																		E7	
																		E8	
																		E11	
																		E5	
																		E12	
PEGs and Salts as a function of pH								PEG 3350 and salts											
3.35K						10K	3.35K												
pH	Ammonium sulfate	Sodium chloride	Lithium sulfate monohydrate	Ammonium acetate	Magnesium Chloride hexahydrate	Ammonium acetate	Mixed chlorides	%	Potassium sodium tartrate tetrahydrate	Sodium malonate pH 7.0	Ammonium citrate tribasic pH 7.0	Succinic acid pH 7.0	Sodium formate	DL-Malic acid pH 7.0	Magnesium formate dihydrate	Zinc acetate dihydrate	Sodium citrate tribasic dihydrate	Potassium thiocyanate	Potassium bromide
5.5	F6	F10	G2	G6	G10	F5		15				H5			H8				
6.5	F7	F11	G3	G7	G11			20	H2	H3	H4		H6	H7		H9	H10		
7.5	F8	F12	G4	G8	G12		F4	25											
8.5	F9	G1	G5	G9	H1			30										H11	H12

Coarse test for chemical conditions likely to produce crystallization

Sherlock and Watson.

“We approached the case, you remember, with an absolutely blank mind, which is always an advantage. We had formed no theories. We were simply there to observe and to draw inferences from our observations”

Sherlock Holmes to Dr. Watson

I never get your limits, Watson. There are unexplored possibilities about you.

Sherlock Holmes on Dr. Watson.

Two pieces of related software under development;

- Sherlock to look at the individual ‘crime’, *i.e.* examine results from a single macromolecule
- Watson to tell the complete story, *i.e.* look at trends from many experiments.



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Microsoft Excel

File Edit View Insert Format Tools Data Window Help Adobe PDF

Arial 8

B I U

4wk sherlock.xls

X000007226 imaged on 08/09/2006 at 19:31

Image of Well #9

Well #9 (6_C0003)
-Precipitate
-Phase Separation
2.38M Ammonium bromide
0.1M Na Citrate, pH: 4

Image of Well #25

Well #25 (6_C0007)
-Clear
1.19M Ammonium bromide
0.1M TAPS, pH: 9

Image of Well #13

Well #13 (6_C0004)
-CRYSTALS
2.38M Ammonium bromide
0.1M HEPES, pH: 7.5

Image of Well #1509

Well #1509 (6_C0570)
-CRYSTALS
-Precipitate
0.1M Magnesium chloride-hexahydrate
0.1M HEPES, pH: 7.5
20%(w/v) PEG 4000

PEG 4000	CAAPS	Na Citrate	Na Acetate	MES	MOPS	HEPES
10	4	5	7	15		
28X	1113	1112	1111			
48X	1114	1113	1112			
48X	1117	1116	1115			
48X	1118	1117	1116			

CONDITION NOT SAMPLED

Decreasing pH leads to crystallization. A large area of space along the crystallization pathway remains un-sampled. There are clear areas to pursue optimization.

Decreasing PEG % leads to crystallization. Again a large area of space along the crystallization pathway remains un-sampled. There are clear areas to pursue optimization.

Chemical Space

Image Filenames

Outcome Summary

AutoShapes

Ready

NUM

Microsoft Excel

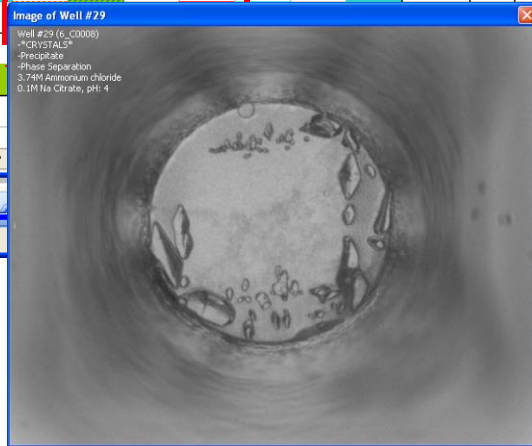
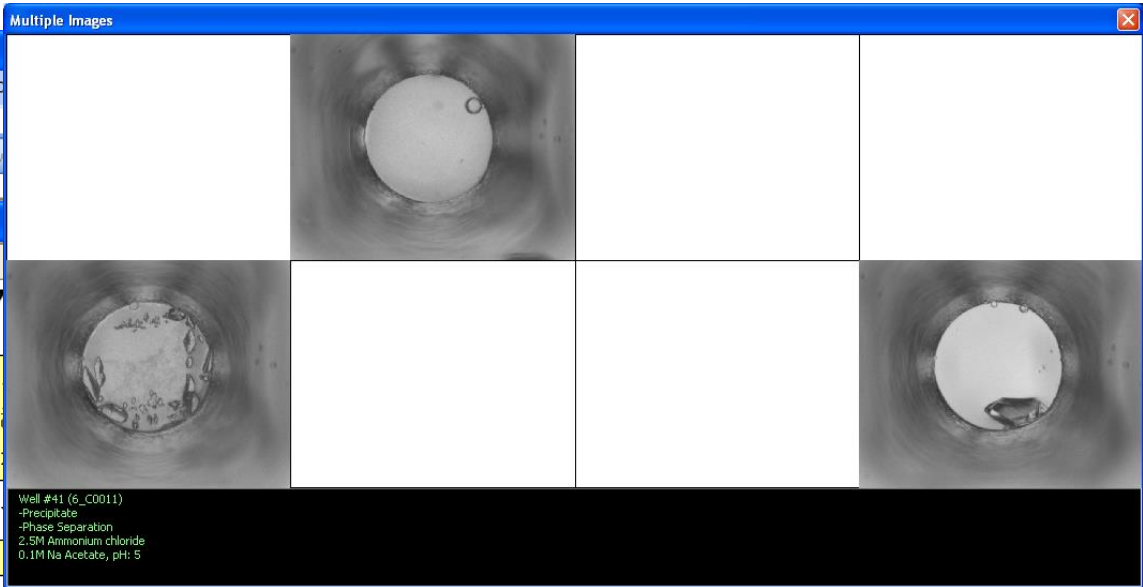
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A2

4wk sherlock.xls

	A	B	C	D
1			X000007	
2			M	CAPS
3		pH		10
4				
5			1.19	
6		bromide	2.38	9
7			3.56	5
8			1.25	
9		chloride	2.5	193



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Sherlock and Watson – Current Status

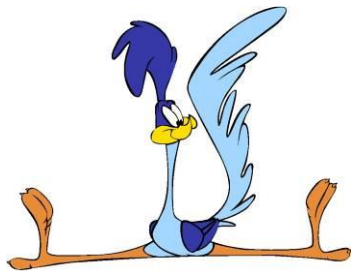
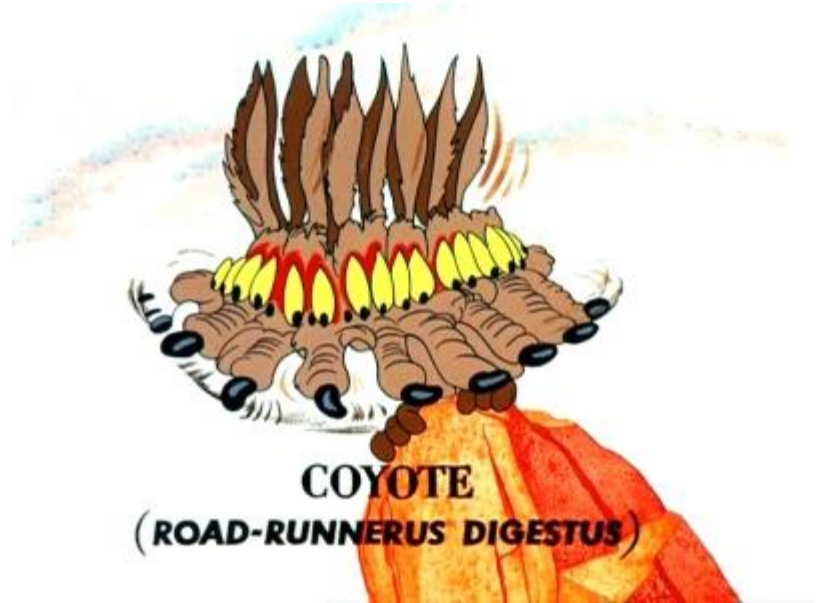
- Sherlock is currently being tested in the High-Throughput laboratory. The aim is to release it to external users as a beta version in the near future.
- There are several possible representations of chemical space available, only one was shown here.
- Currently it requires manual scoring of images. Developments in automated image analysis look very promising and there is near certainty that we can automatically score clear and precipitate images leaving a much smaller number of images to visually examine. Other research is underway to automatically score these as well.
- Watson is under development and at present is only being used by a limited number of testers to analyze the performance of the HWI cocktails and commercial screens used in the laboratory.

Future work

- To automatically flag patterns that may indicate potential regions for further exploration if a crystallization hit does not occur. For example, two results showing clear and precipitate separated by a long un-sampled chemically sensitive pathway.
- To produce separate programs for other screens.
- To incorporate time or temperature resolved data, predict the best optimization strategies or aid the interpretation of current optimization techniques such as Drop Volume Ratio/Temperature (DVR/T) Luft et al., 2007.

How many samples?

In using chemical space mapping to analyze a number of samples it has become clear that 1536 is a good number of experiments to try. It enables a wide range of chemical space to be investigated with sufficient detail to identify common regions for crystallization together with diversely separated regions where different crystal forms may result



It is important to investigate not a single hit but as many hits as you have sample. Visual observation only indicates a crystal, not that it diffracts well or even if it is a macromolecular crystal rather than salt or PEG. Spreading the effort among many hits is better than focusing exclusively on one.



Summary

- No experiment should be considered in isolation.
- In crystallization screening when you have a sparse matrix, incomplete factorial or any other designed sampling of chemical space the results build up a picture of the crystallization landscape.
- An experiment with no crystallization hits that which generates both precipitate and clear conditions is promising when those conditions are separated by an un-sampled chemically sensible direction.
- You should know what crystallization conditions you examined but more importantly how those relate to those that were not sampled.
- Optimize as many samples as you can.
- Check with X-rays as soon as possible.
- The axis of crystallization space have a complex relationship with those in chemical space. We have a limited understanding of those relationships and hopefully Watson will reveal a better story from the >9000 cases we currently have.
- There are many more variables to explore!

Questions?

