Grid

Morpheus™ MD1–46 / MD1–47

A 96 condition 3D protein crystallization screen with original chemistry incorporating a range of low molecular weight ligands found ordered in ~33,000 deposited structures. This unique screen aims to unlock novel chemical space previously inaccessible using conventional screens. Improved to accommodate easy optimisation of conditions*.

MD1-46 is presented as 96 × 10 ml conditions.
MD1-47 is presented as an HT screen with 96 × 1 ml conditions in deep-well blocks.

Features of Morpheus™:

- Simple and effective 3D grid design covering a range of pH, PEGs and salt additives
- Targeted incorporation of 49 low molecular weight ligands observed to promote both initial crystal formation and lattice stability.
- Reduced crystal "stress" - all conditions are cryo-protected.
- Available in both 10ml tube and HT-96 deep well block formats.
- Improved with NEW buffer systems to make optimisation simple.

Introduction

Morpheus is a 96 condition protein crystallization screen with an original chemistry. It is based on extensive data mining of the PDB. The aim is to explore different chemical space than is achieved with conventional screening.

Morpheus incorporates 49 low molecular weight components. They are PDB ligands sharing four main characteristics; they are small (the largest being HEPES MW 238.30 g/mol and the smallest a lithium ion MW 6.94 g/mol), stable, inexpensive and are associated with at least five unrelated PDB structures.

The selection of ligands are listed in Table 1 (data produced on the 14th of July 2008: 35759 structures with ligands in the PDB). Overall the PDB ligands in Morpheus correspond with over 33,000 PDB structures. For instance, the two enantiomers of tartaric acid (PDB ID: TAR and TLA) are found ordered in 113 structures.

Preliminary tests with Morpheus made within the Laboratory of Molecular Biology (LMB)[1] at Cambridge, UK, have shown encouraging results with various targets. In some cases, Morpheus gave hits when all other commercial screens had failed.

Figure 1 shows examples of protein crystallization hits observed while testing Morpheus.

Screen Design

Morpheus is based on a 3D grid design (Figure 2). Thirty of the top PDB ligands from Table 1 are grouped into eight mixes of additives depending on their chemical class (e.g. alcohols, carboxylic acids, etc) (Table 2).

These top PDB ligands also happen to be "biological buffers" like HEPES (PDB ID: EPE, 201 hits) and have been used to build three buffer systems*. Each buffer system includes different buffers with close pKa's (Table 3).

There are nine precipitants included in the composition of Morpheus. They are grouped into four mixes of precipitants (Table 4). The main characteristic of the four mixes is that they contain at least a PEG (Polyethylene glycol) and a different type of precipitant that is also a cryo-agent (e.g. Glycerol). All the conditions of Morpheus are cryo-protected to minimize further mechanical stress on the crystals.

Each mix of precipitants is systematically tested with all the mixes of additives and the mixes of buffers. The proportions of stocks are always the same for making any condition of the three-dimensional grid: 5:1:1:3 of precipitants, ligands, buffers and water respectively. When almost a third of the composition is water, there is space for making an optimization screen with higher concentration of any mix/component.

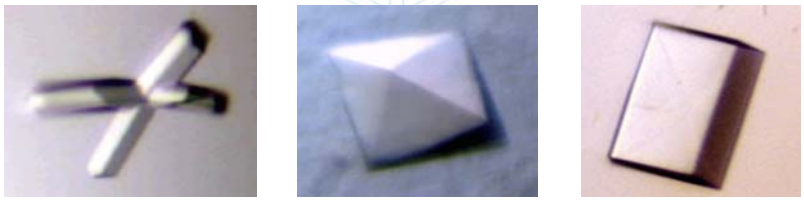

Figure 1. Examples of successful crystallization with Morpheus
(with the permission of Pobbati A., Low H. and Berndt A.)

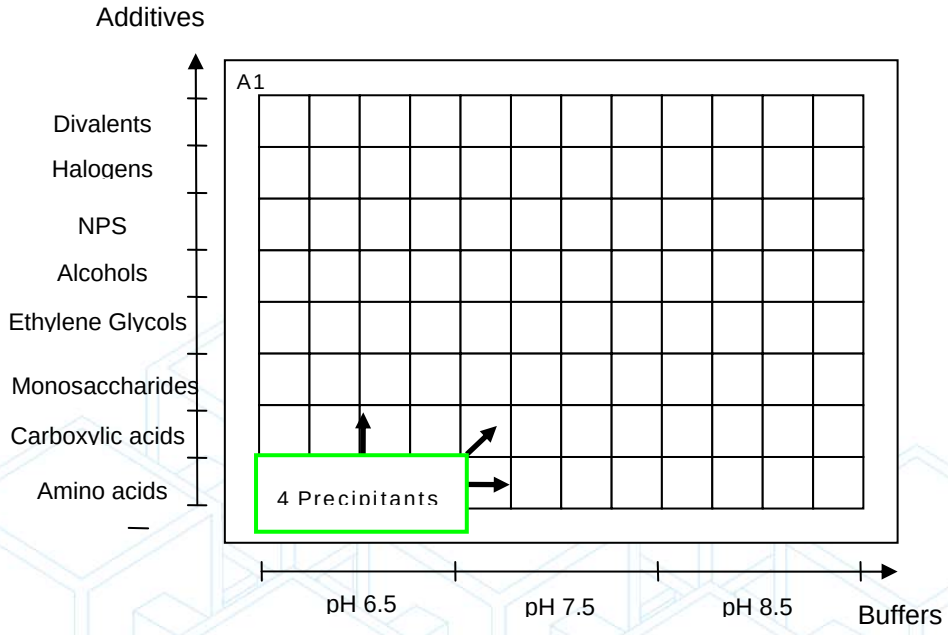

Figure 2. Schematic of Morpheus™ – A three-dimensional grid screen

***Optimisation is now easily achieved because of the improvements made to the buffer systems used in Morpheus. The user now has complete control over what pH they would like to optimise at. The original Buffer Mixes will still be available to purchase from Molecular Dimensions Ltd.**

Formulation notes

The reagents in Morpheus are formulated using ultrapure water (>18.0 MΩ) and are sterile-filtered using 0.22 μm filters. No preservatives are added.

Final pH may vary from that specified on the datasheet.

Contact Us

Individual reagents and stock solutions for optimization are available from Molecular Dimensions.

Molecular Dimensions Ltd. would be very grateful if investigators were prepared to provide feedback on their own experiences with the new screen. Crystallization reports or pictures can be e-mailed to: enquiries@moleculardimensions.com

Contact and product details can be found at www.moleculardimensions.com

References

1. D. Stock, O. Perisic, J. Löwe "Robotic nanolitre protein crystallisation at the LMB", Prog. Biophys. Mol. Biol. 88 (2005) 311-32See also LMB-Cambridge robotic crystallisation web pages: http://www2.mrc-lmb.cam.ac.uk/groups/JYL/WWWrobots/

Table 1: List of PDB ligands in Morpheus

PDB Ligand name(s)	Class	PDB ID(s)	Structure Hits*
1,2-Ethanediol (ethylene glycol)	Precipitant	EDO, EGL	1081
1,2-Propanediol (enantiomers R and S)	Alcohols	PGO, PGR	41
1,3-Propanediol	Alcohols	PDO	7
1,4-Butanediol	Alcohols	BU1	11
1,6-Hexanediol	Alcohols	HEZ	19
1-Butanol	Alcohols	1BO	7
2-(N-Morpholino)-ethane sulfonic acid (MES)	Buffer	MES	315
2-Amino-2-hydroxymethyl-propane-1,3-diol (Tris)	Buffer	TRS	334
2-Methyl-2,4-pentanediol (MPD, enantiomers R and S)	Precipitant	MPD, MRD	504
3-Morpholinopropane-1-sulfonic acid (MOPS)	Buffer	MPO	21
4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)	Buffer	EPE	201
Acetic acid, acetate, acetyl	Carboxylic acids	ACY, ACT, ACE	1890
(*S*)-2-Aminopropanoic acid (Alanine, (enantiomers L and D)	Amino acids	ALA, DAL	35
Amino, Ammonia, Ammonium	multiple	NH2, NH3, NH4	582
N,N-bis(2-hydroxyethyl)glycine (Bicine)	Buffer	BCN	13
Bromide	Halogens	BR	120
Calcium	Divalents	CA	3959
Chloride	Multiple	CL	2842
Citric acid, citrate	Carboxylic acids	CIT, FLC	384
D-Galactose (anomers α and β)	Monosaccharides	GAL, GLA	86
D-Glucose (anomers α and β)	Monosaccharides	GLC, BGC	206
Di(Hydroxyethyl)ether (Di-Ethyleneglycol)	Ethylene glycols	PEG	209
D-Mannose (anomers α and β)	Monosaccharides	MAN, BMA	178
D-Xylopyranose (anomers α and β)	Monosaccharides	XYL, XYP	41
Fluoride	Halogens	F	16
Formic acid	Carboxylic acids	FMT	267
Glycerol	Amino acids	GOL	2884
Glycine	Buffer	GLY	50
Imidazole	Halogens	IMD	154
Iodide	Alcohols	IOD	178
Isopropyl alcohol (iso-propanol, 2-Propanol)	Monosaccharides	IPA, IOH	174
L-Fucose (anomers α and β)	Amino acids	FUC, FUL	62
L-Glutamic acid	Precipitant	GLU	66
Lysine (enantiomers L and D)	Amino acids	LYS, DLY	36
Magnesium	Divalents	MG	3991
N-Acetyl-d-glucosamine (anomers α and β)	Monosaccharides	NAG,NBG	1150
Nitrate	NPS	NO3	156
Oxamic acid	Carboxylic acids	OXM	17
Penta(hydroxyethyl)ether (Penta-Ethyleneglycol)	Ethylene glycols	1PE	91
Phosphates	NPS	PO4, PI, 2HP	1687
Potassium	Carboxylic acids	K	720
Serine (enantiomers L and D)	Amino acids	SER, DSN	38
Sodium	multiple	NA	1926
Sulfate	NPS	SO4	5793
Tartaric acid (enantiomers R and S)	Carboxylic acids	TAR, TLA	113
Tetra(hydroxyethyl)ether (Tetra-Ethyleneglycol)	Ethylene glycols	PG4	194
Tri(Hydroxyethyl)ether (Tri-Ethyleneglycol)	Ethylene glycols	PGE	107
* as of July 2008.		**SUM**	32956

Table 2: Mixes of additives used in Morpheus™

Mix name	Composition	Catalogue Number (100 & 250mL)
Divalents	$MgCl_2$; $CaCl_2$	MD2-100(250)-70
Halogens	NaF; NaBr; NaI	MD2-100(250)-71
NPS†	$NaNO_3$; Na_2HPO_4; $(NH_4)_2SO_4$	MD2-100(250)-72
Alcohols	1,6-Hexanediol; 1-Butanol 1,2-Propanediol (racemic); 2-Propanol; 1,4-Butanediol; 1,3-Propanediol	MD2-100(250)-73
Ethylene glycols	Di-Ethyleneglycol; Tri-Ethyleneglycol; Tetra-Ethyleneglycol; Penta-Ethyleneglycol	MD2-100(250)-74
Monosaccharides	D-Glucose; D-Mannose; D-Galactose; L-Fucose; D-Xylose; N-Acetyl-D-Glucosamine	MD2-100(250)-75
Carboxylic acids	Na-Formate; NH_4-Acetate; Na_3-Citrate; NaK-Tartrate (racemic); Na-Oxamate	MD2-100(250)-76
Amino acids	L-Na-Glutamate; Alanine (racemic); Glycine; Lysine HCl (racemic); Serine (racemic)	MD2-100(250)-77

†**NPS;** Nitrate Phosphate Sulfate.

Table 3: Buffer systems* used in Morpheus™

Mix name	Conc.	pH @ 20°C	Composition	Catalogue Number (100 & 250mL)
Buffer System 1	1.0M	6.5	Imidazole;; MES (acid);	MD2-100(250)-100
Buffer System 2	1.0M	7.5	Sodium HEPES; MOPS (acid)	MD2-100(250)-101
Buffer System 3	1.0M	8.5	Tris (base); Bicine	MD2-100(250)-102

***These buffers have been improved to aid optimisation. The buffers in each system are un-titrated and mixed together to give the appropriate pH. Each Buffer system is available as an individual kit for your convenience (a Buffer composition table for these systems are available from our Downloads Centre).**

Table 4: Mixes of Precipitants used in Morpheus™

Mix name	Conc.	Composition	Catalogue Number (100 & 250mL)
P550MME_P20K	60%	PEGMME 550; PEG 20K	MD2-100(250)-81
EDO_P8K	60%	Ethylene glycol; PEG 8K	MD2-100(250)-82
GOL_P4K	60%	Glycerol; PEG 4K	MD2-100(250)-83
MPD_P1K_P3350	75%	MPD (racemic); PEG 1K; PEG 3350	MD2-100(250)-84

The additive and precipitant mixes are available to buy from Molecular Dimensions Ltd.
For individual components or customised mixes please contact us directly at enquiries@moleculardimensions.com

Table 5- MD1-46 (MD1-47 HT) Morpheus™ Conditions 1-48 (A1-D12)

Well	Tube	Ligand stock	Conc.	Buffer System	Conc.	pH @ (20°C)	precipitant stock	Conc.
A1	1-1	Divalents	0.06M	1	0.1M	6.5	P550MME_P20K	30.0%
A2	1-2	Divalents	0.06M	1	0.1M	6.5	EDO_P8K	30.0%
A3	1-3	Divalents	0.06M	1	0.1M	6.5	GOL_P4K	30.0%
A4	1-4	Divalents	0.06M	1	0.1M	6.5	MPD_P1K_P3350	37.5%
A5	1-5	Divalents	0.06M	2	0.1M	7.5	P550MME_P20K	30.0%
A6	1-6	Divalents	0.06M	2	0.1M	7.5	EDO_P8K	30.0%
A7	1-7	Divalents	0.06M	2	0.1M	7.5	GOL_P4K	30.0%
A8	1-8	Divalents	0.06M	2	0.1M	7.5	MPD_P1K_P3350	37.5%
A9	1-9	Divalents	0.06M	3	0.1M	8.5	P550MME_P20K	30.0%
A10	1-10	Divalents	0.06M	3	0.1M	8.5	EDO_P8K	30.0%
A11	1-11	Divalents	0.06M	3	0.1M	8.5	GOL_P4K	30.0%
A12	1-12	Divalents	0.06M	3	0.1M	8.5	MPD_P1K_P3350	37.5%
B1	1-13	Halogens	0.09M	1	0.1M	6.5	P550MME_P20K	30.0%
B2	1-14	Halogens	0.09M	1	0.1M	6.5	EDO_P8K	30.0%
B3	1-15	Halogens	0.09M	1	0.1M	6.5	GOL_P4K	30.0%
B4	1-16	Halogens	0.09M	1	0.1M	6.5	MPD_P1K_P3350	37.5%
B5	1-17	Halogens	0.09M	2	0.1M	7.5	P550MME_P20K	30.0%
B6	1-18	Halogens	0.09M	2	0.1M	7.5	EDO_P8K	30.0%
B7	1-19	Halogens	0.09M	2	0.1M	7.5	GOL_P4K	30.0%
B8	1-20	Halogens	0.09M	2	0.1M	7.5	MPD_P1K_P3350	37.5%
B9	1-21	Halogens	0.09M	3	0.1M	8.5	P550MME_P20K	30.0%
B10	1-22	Halogens	0.09M	3	0.1M	8.5	EDO_P8K	30.0%
B11	1-23	Halogens	0.09M	3	0.1M	8.5	GOL_P4K	30.0%
B12	1-24	Halogens	0.09M	3	0.1M	8.5	MPD_P1K_P3350	37.5%
C1	1-25	NPS	0.09M	1	0.1M	6.5	P550MME_P20K	30.0%
C2	1-26	NPS	0.09M	1	0.1M	6.5	EDO_P8K	30.0%
C3	1-27	NPS	0.09M	1	0.1M	6.5	GOL_P4K	30.0%
C4	1-28	NPS	0.09M	1	0.1M	6.5	MPD_P1K_P3350	37.5%
C5	1-29	NPS	0.09M	2	0.1M	7.5	P550MME_P20K	30.0%
C6	1-30	NPS	0.09M	2	0.1M	7.5	EDO_P8K	30.0%
C7	1-31	NPS	0.09M	2	0.1M	7.5	GOL_P4K	30.0%
C8	1-32	NPS	0.09M	2	0.1M	7.5	MPD_P1K_P3350	37.5%
C9	1-33	NPS	0.09M	3	0.1M	8.5	P550MME_P20K	30.0%
C10	1-34	NPS	0.09M	3	0.1M	8.5	EDO_P8K	30.0%
C11	1-35	NPS	0.09M	3	0.1M	8.5	GOL_P4K	30.0%
C12	1-36	NPS	0.09M	3	0.1M	8.5	MPD_P1K_P3350	37.5%
D1	1-37	Alcohols	0.12M	1	0.1M	6.5	P550MME_P20K	30.0%
D2	1-38	Alcohols	0.12M	1	0.1M	6.5	EDO_P8K	30.0%
D3	1-39	Alcohols	0.12M	1	0.1M	6.5	GOL_P4K	30.0%
D4	1-40	Alcohols	0.12M	1	0.1M	6.5	MPD_P1K_P3350	37.5%
D5	1-41	Alcohols	0.12M	2	0.1M	7.5	P550MME_P20K	30.0%
D6	1-42	Alcohols	0.12M	2	0.1M	7.5	EDO_P8K	30.0%
D7	1-43	Alcohols	0.12M	2	0.1M	7.5	GOL_P4K	30.0%
D8	1-44	Alcohols	0.12M	2	0.1M	7.5	MPD_P1K_P3350	37.5%
D9	1-45	Alcohols	0.12M	3	0.1M	8.5	P550MME_P20K	30.0%
D10	1-46	Alcohols	0.12M	3	0.1M	8.5	EDO_P8K	30.0%
D11	1-47	Alcohols	0.12M	3	0.1M	8.5	GOL_P4K	30.0%
D12	1-48	Alcohols	0.12M	3	0.1M	8.5	MPD_P1K_P3350	37.5%

Table 5 contd. - MD1-46 (MD1-47 HT) Morpheus™ Conditions 49-96 (E1-H12)

Well	Tube	Ligand stock	Conc.	Buffer System	Conc.	pH @ (20°C)	Precipitant stock	Conc.
E1	2-1	Ethylene Glycols	0.12M	1	0.1M	6.5	P550MME_P20K	30.0%
E2	2-2	Ethylene Glycols	0.12M	1	0.1M	6.5	EDO_P8K	30.0%
E3	2-3	Ethylene Glycols	0.12M	1	0.1M	6.5	GOL_P4K	30.0%
E4	2-4	Ethylene Glycols	0.12M	1	0.1M	6.5	MPD_P1K_P3350	37.5%
E5	2-5	Ethylene Glycols	0.12M	2	0.1M	7.5	P550MME_P20K	30.0%
E6	2-6	Ethylene Glycols	0.12M	2	0.1M	7.5	EDO_P8K	30.0%
E7	2-7	Ethylene Glycols	0.12M	2	0.1M	7.5	GOL_P4K	30.0%
E8	2-8	Ethylene Glycols	0.12M	2	0.1M	7.5	MPD_P1K_P3350	37.5%
E9	2-9	Ethylene Glycols	0.12M	3	0.1M	8.5	P550MME_P20K	30.0%
E10	2-10	Ethylene Glycols	0.12M	3	0.1M	8.5	EDO_P8K	30.0%
E11	2-11	Ethylene Glycols	0.12M	3	0.1M	8.5	GOL_P4K	30.0%
E12	2-12	Ethylene Glycols	0.12M	3	0.1M	8.5	MPD_P1K_P3350	37.5%
F1	2-13	Monosaccharides	0.12M	1	0.1M	6.5	P550MME_P20K	30.0%
F2	2-14	Monosaccharides	0.12M	1	0.1M	6.5	EDO_P8K	30.0%
F3	2-15	Monosaccharides	0.12M	1	0.1M	6.5	GOL_P4K	30.0%
F4	2-16	Monosaccharides	0.12M	1	0.1M	6.5	MPD_P1K_P3350	37.5%
F5	2-17	Monosaccharides	0.12M	2	0.1M	7.5	P550MME_P20K	30.0%
F6	2-18	Monosaccharides	0.12M	2	0.1M	7.5	EDO_P8K	30.0%
F7	2-19	Monosaccharides	0.12M	2	0.1M	7.5	GOL_P4K	30.0%
F8	2-20	Monosaccharides	0.12M	2	0.1M	7.5	MPD_P1K_P3350	37.5%
F9	2-21	Monosaccharides	0.12M	3	0.1M	8.5	P550MME_P20K	30.0%
F10	2-22	Monosaccharides	0.12M	3	0.1M	8.5	EDO_P8K	30.0%
F11	2-23	Monosaccharides	0.12M	3	0.1M	8.5	GOL_P4K	30.0%
F12	2-24	Monosaccharides	0.12M	3	0.1M	8.5	MPD_P1K_P3350	37.5%
G1	2-25	Carboxylic acids	0.10M	1	0.1M	6.5	P550MME_P20K	30.0%
G2	2-26	Carboxylic acids	0.10M	1	0.1M	6.5	EDO_P8K	30.0%
G3	2-27	Carboxylic acids	0.10M	1	0.1M	6.5	GOL_P4K	30.0%
G4	2-28	Carboxylic acids	0.10M	1	0.1M	6.5	MPD_P1K_P3350	37.5%
G5	2-29	Carboxylic acids	0.10M	2	0.1M	7.5	P550MME_P20K	30.0%
G6	2-30	Carboxylic acids	0.10M	2	0.1M	7.5	EDO_P8K	30.0%
G7	2-31	Carboxylic acids	0.10M	2	0.1M	7.5	GOL_P4K	30.0%
G8	2-32	Carboxylic acids	0.10M	2	0.1M	7.5	MPD_P1K_P3350	37.5%
G9	2-33	Carboxylic acids	0.10M	3	0.1M	8.5	P550MME_P20K	30.0%
G10	2-34	Carboxylic acids	0.10M	3	0.1M	8.5	EDO_P8K	30.0%
G11	2-35	Carboxylic acids	0.10M	3	0.1M	8.5	GOL_P4K	30.0%
G12	2-36	Carboxylic acids	0.10M	3	0.1M	8.5	MPD_P1K_P3350	37.5%
H1	2-37	Amino acids	0.10M	1	0.1M	6.5	P550MME_P20K	30.0%
H2	2-38	Amino acids	0.10M	1	0.1M	6.5	EDO_P8K	30.0%
H3	2-39	Amino acids	0.10M	1	0.1M	6.5	GOL_P4K	30.0%
H4	2-40	Amino acids	0.10M	1	0.1M	6.5	MPD_P1K_P3350	37.5%
H5	2-41	Amino acids	0.10M	2	0.1M	7.5	P550MME_P20K	30.0%
H6	2-42	Amino acids	0.10M	2	0.1M	7.5	EDO_P8K	30.0%
H7	2-43	Amino acids	0.10M	2	0.1M	7.5	GOL_P4K	30.0%
H8	2-44	Amino acids	0.10M	2	0.1M	7.5	MPD_P1K_P3350	37.5%
H9	2-45	Amino acids	0.10M	3	0.1M	8.5	P550MME_P20K	30.0%
H10	2-46	Amino acids	0.10M	3	0.1M	8.5	EDO_P8K	30.0%
H11	2-47	Amino acids	0.10M	3	0.1M	8.5	GOL_P4K	30.0%
H12	2-48	Amino acids	0.10M	3	0.1M	8.5	MPD_P1K_P3350	37.5%