Protein Crystallization: Simply *grab* the needle from the haystack

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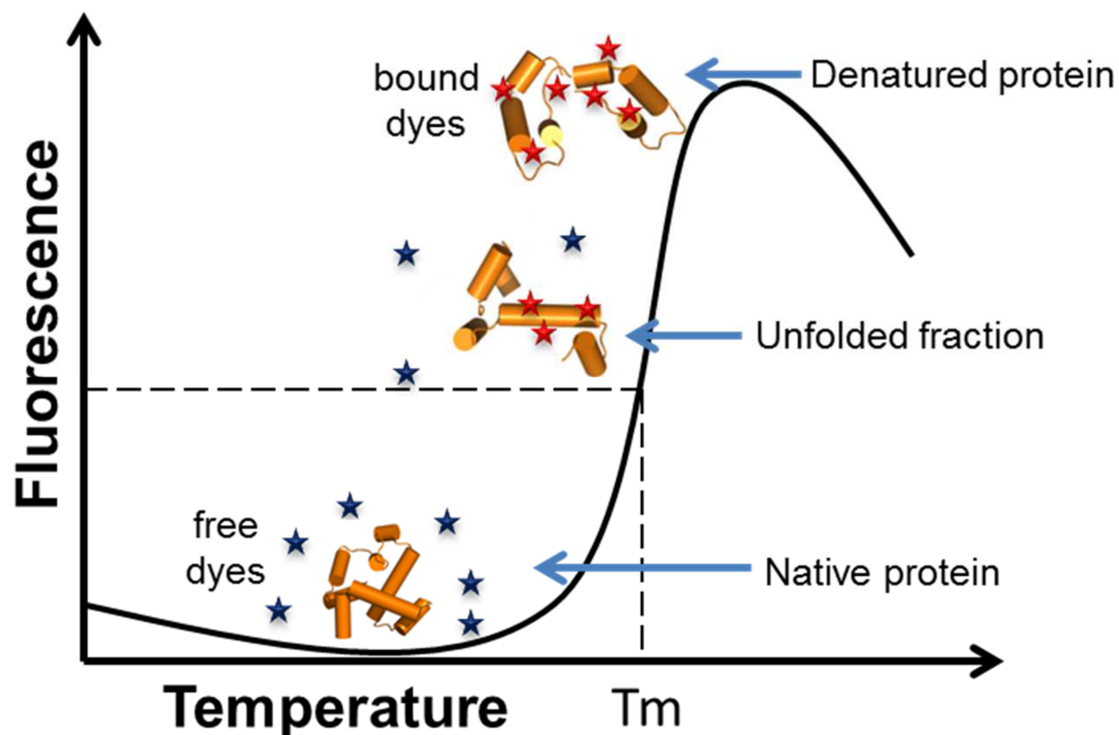
Agenda

1. JBScreen Thermofluor
2. Membrane Proteins: JBScreen LCP & LCP Lipids
3. Frag Xtal Screen

Crystallizability depends on a protein's thermostability[1,2]



JBScreen Thermofluor determines thermostability as a protein's melting temperature (Tm)

(1) Ericsson *et al.* (2006) Thermofluor-based high-throughput stability optimization of proteins for structural studies. *Anal. Biochem.* 357(2):289.
(2) Dupeux *et al.* (2011) A thermal stability assay can help to estimate the crystallization likelihood of biological samples. *Acta Cryst. D* 67:915.

A protein's thermostability is affected by FUNDAMENTAL and SPECIFIC buffer components

FUNDAMENTAL FACTORS
affect
the whole protein molecule

- **Proton concentration (i.e. pH):**
  Determines protein net charge
- **Ionic strength:**
  Influences size of hydration shells

SPECIFIC FACTORS
affect
energetically important hot spots on the protein surface

- **Salt:**
  Ions with valency-specific effects on distinct sites
- **Additives:**
  Cofactors/Ligands that interact with active site

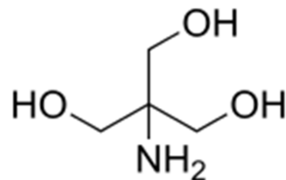
How to screen for thermostability systematically …?

Conventional buffers do not allow an independent pH-screen…
…since pH, ionic strength and additives are interdependent variables

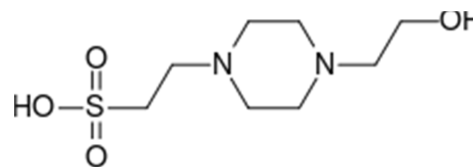
Buffer Solution = Multi-Parameter System of

- pH
- Ionic Strength
- Chemistry (specific ions & additives)

Same pH…



50 mM TRIS/HCl pH 8.0

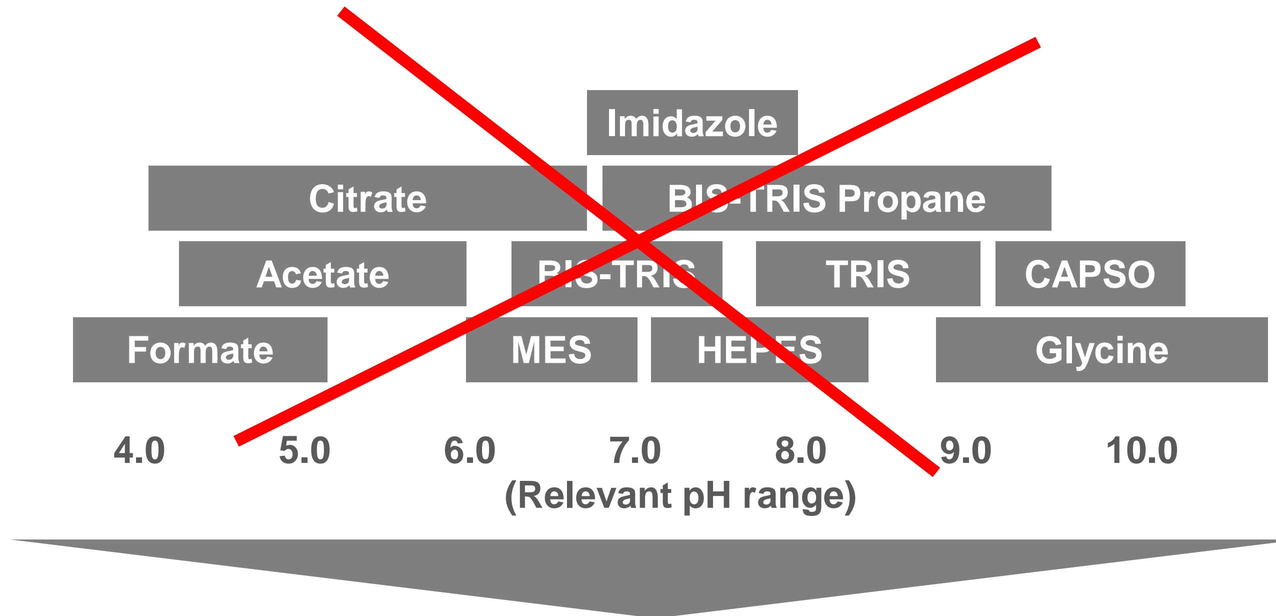


50 mM HEPES/NaOH pH 8.0

…but different chemistry

Conventional Screening inevitably changes multiple parameters simultaneously

Solution: JBScreen Thermofluor is based on "Super Buffers"[1]

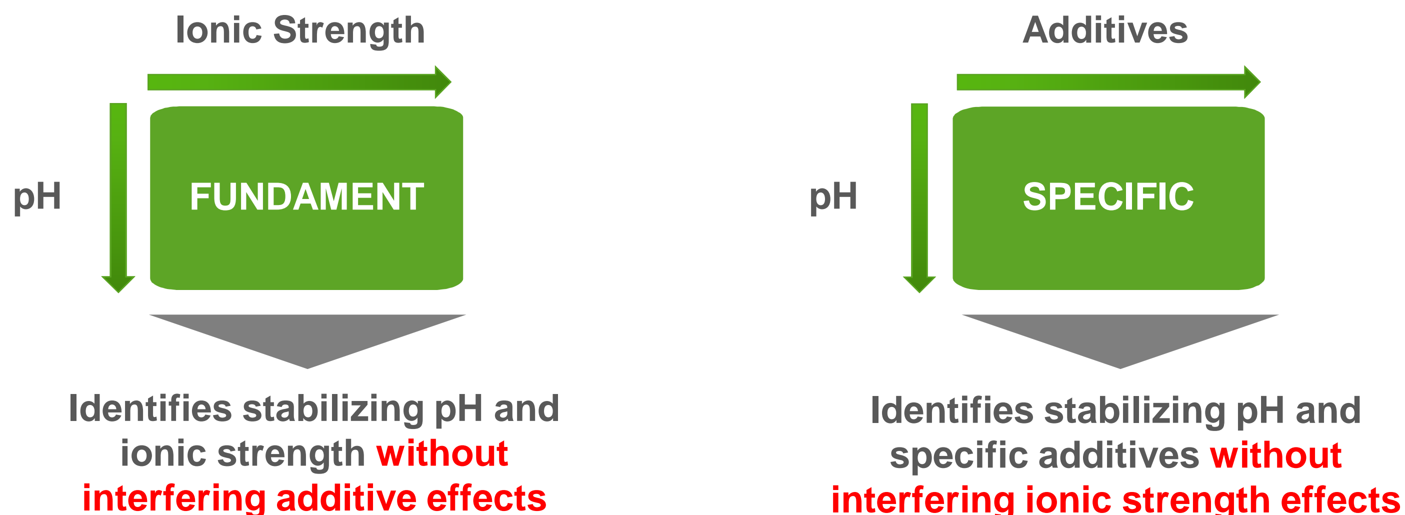


Super Buffer

- Composed of a mixture of three individual buffers with distinct pK_a values
- Specific ratio of individual buffers
- Cover the entire pH range from 4.0 – 10.0
- Maintain a constant chemical environment for screening

(1) Newman (2004) Novel buffer systems for macromolecular crystallization. *Acta Cryst. D* 60:610.

With Super-Buffer based JBScreen Thermofluor FUNDAMENT and SPECIFIC simply grab for the needle in the haystack



Identifies stabilizing pH and ionic strength **without interfering additive effects**

Identifies stabilizing pH and specific additives **without interfering ionic strength effects**

Optimization / Refinement

- Different Buffers @ optimum pH (JBScreen Buffers & Buffers Xtreme)
- Concentration of specific ion(s)

Small money and time with JBScreen Thermofluor may safe big money and time during crystallization!

Screen	Cat.No.
JBScreen Thermofluor FUNDAMENT → pure pH effect at various ionic strengths	CS-332
JBScreen Thermofluor SPECIFIC → additive effects using high-scoring mono, di- and trivalent ions[1]	CS-333
JBScreen Buffers → common buffers @ neutral pH	CS-214
JBScreen Buffers Xtreme → common buffers @ extreme pH	CS-215

JBScreen Thermofluor is the first commercially available systematic protein stability screen

(1) http://www.rcsb.org/pdb/home/home.do

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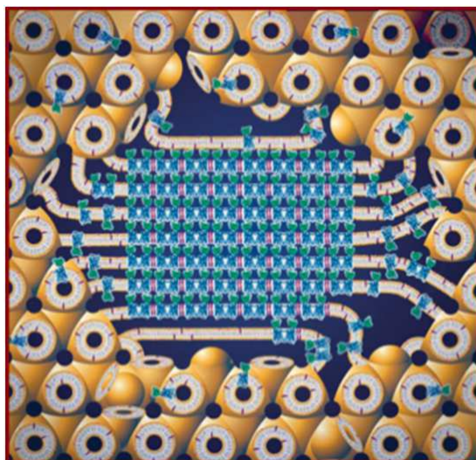
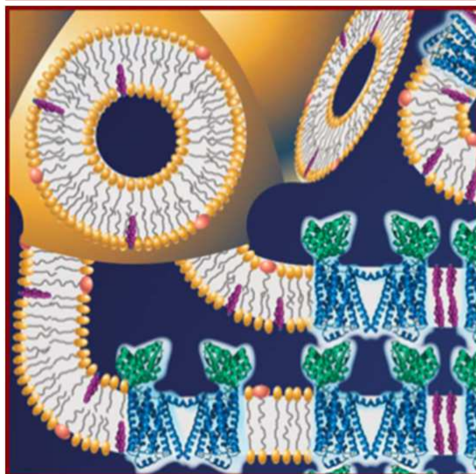
JBScreen LCP is combined from the latest successful conditions





192 integral membrane proteins[1] were analyzed from the pdb[2], evaluated and arranged to the novel JBScreen LCP:

- 96 conditions
- Structured by main precipitant
- Elimination of cacodylate and other highly toxic chemicals
- Elimination of metastable conditions

Product	Cat.No.
JBScreen LCP 4 x 24 solutions (10 ml each)	CS-340
JBScreen LCP HTS 96 solutions (1,7 ml each)	CS-213L

(1) Caffrey (2015) A comprehensive review of the lipid cubic phase or *in meso* method for crystallizing membrane and soluble proteins and complexes. *Acta Cryst F* 71:3

(2) http://www.rcsb.org/pdb/home/home.do

LCP Lipids: There is more than Monoolein[1,2]

Cat. No.	Common Name	Abbreviation	Lipid#	C–C double bond
X-LCP-101	Monoolein	9.9 MAG	C18:1	9 cis
X-LCP-102	Monopalmitolein	9.7 MAG	C16:1	9 cis
X-LCP-103	Monovaccenin	11.7 MAG	C18:1	11 cis
X-LCP-104	Monoeicosenoin	11.9 MAG	C20:1	11 cis

Change LCP parameters

- Bilayer thickness
- Bending elasticity
- Packaging
- Polarity

Crystallization in LCP: Screen for the optimum condition and the optimum LCP Lipid

(1) Caffrey (2015) A comprehensive review of the lipid cubic phase or *in meso* method for crystallizing membrane and soluble proteins and complexes. *Acta Cryst F* 71:3

(2) Caffrey and Cherezov (2009) Crystallizing Membrane Proteins Using Lipidic Mesophases. *Nat Protoc.* 4(5):706.

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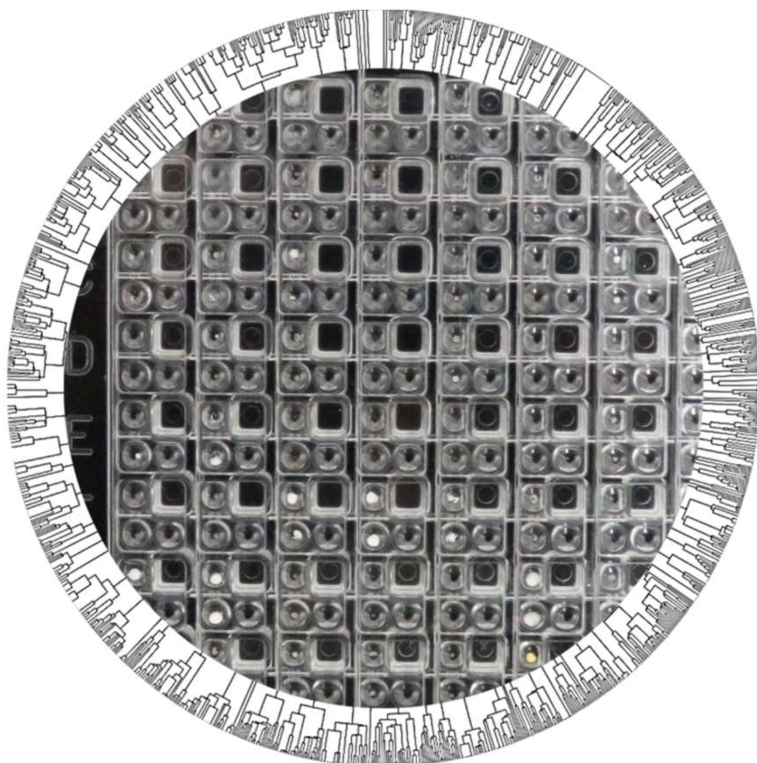
Frag Xtal Screen is coming soon



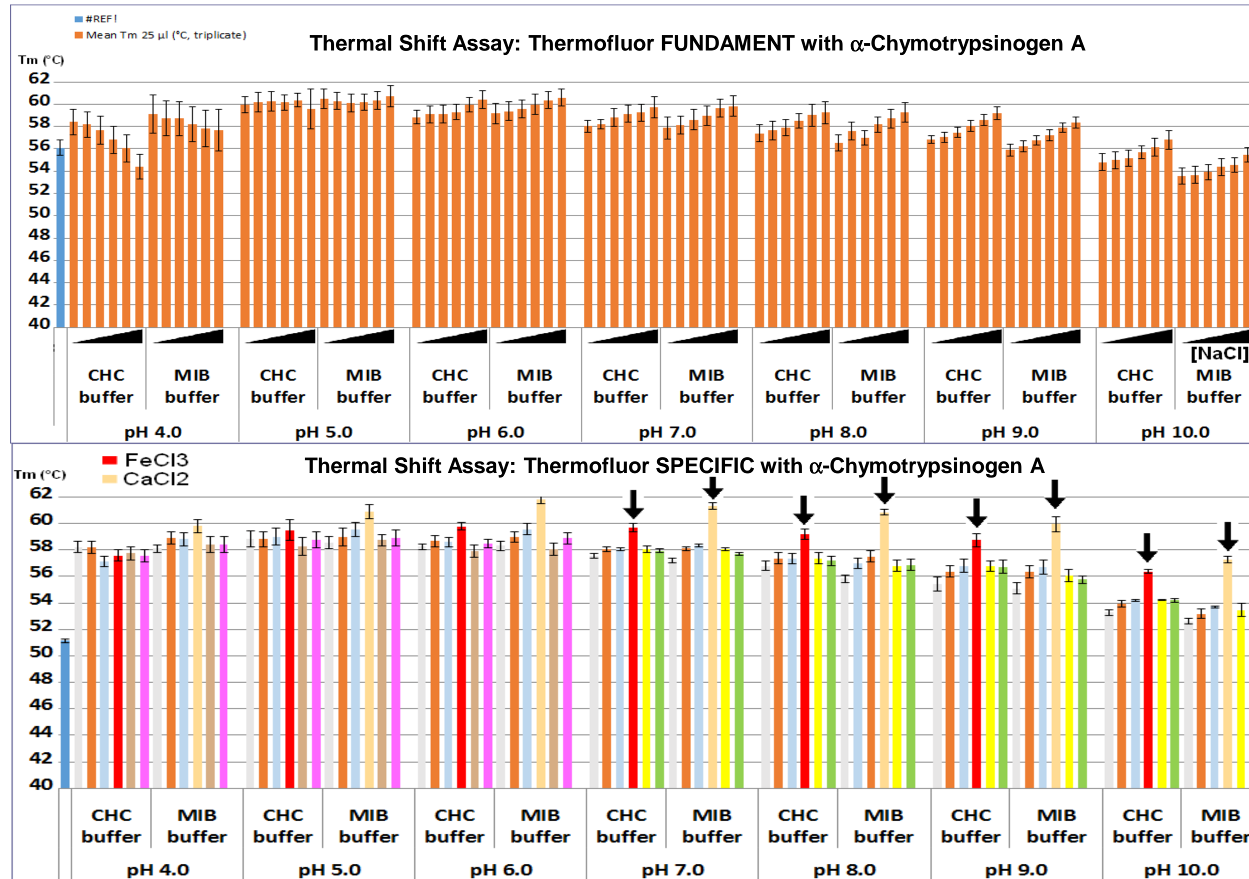
- 96 solid fragments
- Provided in 3 well crystallization plate
- Designed for crystal soaking
- Direct and easy crystallographic analysis of fragment binding(1-3)

(1) Huschmann *et al.* (2016) Structures of endothiapepsin-fragment complexes from crystallographic fragment screening using a novel, diverse and affordable 96-compound fragment library. *Acta Cryst F* 72:346.
(2) Schiebel *et al.* (2016) Six Biophysical Screening Methods Miss a Large Proportion of Crystallographic Discovered Fragment Hits: A Case Study. *ACS Chem. Biol.* 11:1693.
(3) Schiebel *et al.* (2015) One Question, Multiple Answers: Biochemical and Biophysical Screening Methods Retrieve Deviating Fragment Hit Lists. *ChemMedChem* 10:1511.

Our Xtal group is very pleased to answer your inquiries!
xtals@jenabioscience.com

Backups

Stabilizing effects on α-Chymotrypsinogen are directly correlated with crystallizability



FUNDAMENT:
stable from pH 5-8; stability dependent on ionic strength at pH>6

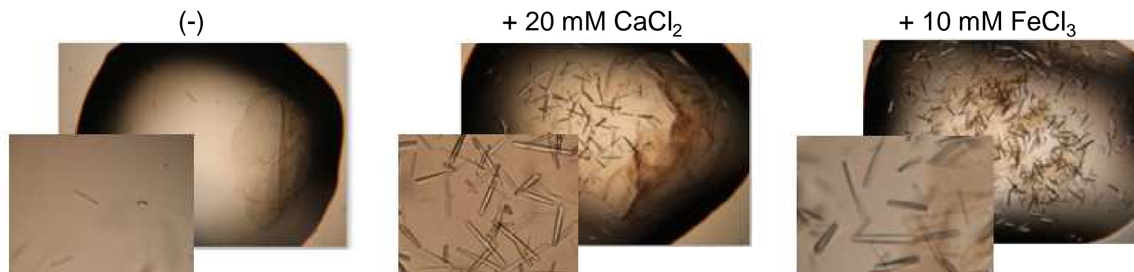
SPECIFIC:
stabilization is induced by either $FeCl_3$ or $CaCl_2$ (iron-binding properties are not described so far)

Buffer for crystallization

20 mg/ml α-Chymotrypsinogen in 20 mM HEPES (pH 7.0), 500 mM NaCl

Droplets with $CaCl_2$ and $FeCl_3$

(-) + 20 mM $CaCl_2$ + 10 mM $FeCl_3$



Crystallization: 100 mM Phosphate (pH 7.5), 20% PEG 3350, 25% PEG 400

Crystal growth is promoted by $CaCl_2$ and $FeCl_3$

Apply JBScreen Thermofluor for hydrophobic targets

Hydrophobic protein target

- Apply the intrinsic red shift of tryptophan residues (e.g. in the Prometheus machine from NanoTemper)
- Apply the new thiol-specific dye (JBS, in development)

Little protein

- Apply 5 µl total assay volume instead of 25 µl and save 80% of protein

Promising approach for membrane proteins, ligands, …

JBScreen Thermofluor FUNDAMENT covers pH 4-10 and ionic strength 0…1,000 mM

	1	2	3	4	5	6	7	8	9	10	11	12
A	CP + Dye CB-1	CP + Dye CB-1	CP + Dye CB-1	CP + Dye CB-2	CP + Dye CB-2	CP + Dye CB-2	CP + Dye CB-3	CP + Dye CB-3	CP + Dye CB-3	TP + Dye Original Buffer	TP + Dye Original Buffer	TP + Dye Original Buffer
B	4.0	4.0 50 mM NaCl	4.0 125 mM NaCl	4.0 250 mM NaCl	4.0 500 mM NaCl	4.0 1 M NaCl	4.0	4.0 50 mM NaCl	4.0 125 mM NaCl	4.0 250 mM NaCl	4.0 500 mM NaCl	4.0 1 M NaCl
C	5.0	5.0 50 mM NaCl	5.0 125 mM NaCl	5.0 250 mM NaCl	5.0 500 mM NaCl	5.0 1 M NaCl	5.0	5.0 50 mM NaCl	5.0 125 mM NaCl	5.0 250 mM NaCl	5.0 500 mM NaCl	5.0 1 M NaCl
D	6.0	6.0 50 mM NaCl	6.0 125 mM NaCl	6.0 250 mM NaCl	6.0 500 mM NaCl	6.0 1 M NaCl	6.0	6.0 50 mM NaCl	6.0 125 mM NaCl	6.0 250 mM NaCl	6.0 500 mM NaCl	6.0 1 M NaCl
E	7.0	7.0 50 mM NaCl	7.0 125 mM NaCl	7.0 250 mM NaCl	7.0 500 mM NaCl	7.0 1 M NaCl	7.0	7.0 50 mM NaCl	7.0 125 mM NaCl	7.0 250 mM NaCl	7.0 500 mM NaCl	7.0 1 M NaCl
F	8.0	8.0 50 mM NaCl	8.0 125 mM NaCl	8.0 250 mM NaCl	8.0 500 mM NaCl	8.0 1 M NaCl	8.0	8.0 50 mM NaCl	8.0 125 mM NaCl	8.0 250 mM NaCl	8.0 500 mM NaCl	8.0 1 M NaCl
G	9.0	9.0 50 mM NaCl	9.0 125 mM NaCl	9.0 250 mM NaCl	9.0 500 mM NaCl	9.0 1 M NaCl	9.0	9.0 50 mM NaCl	9.0 125 mM NaCl	9.0 250 mM NaCl	9.0 500 mM NaCl	9.0 1 M NaCl
H	10.0	10.0 50 mM NaCl	10.0 125 mM NaCl	10.0 250 mM NaCl	10.0 500 mM NaCl	10.0 1 M NaCl	10.0	10.0 50 mM NaCl	10.0 125 mM NaCl	10.0 250 mM NaCl	10.0 500 mM NaCl	10.0 1 M NaCl

pH

100 mM Super Buffer 1 — Ionic Strength

100 mM Super Buffer 2 — Ionic Strength

Super Buffer 1: composed of Citric acid, HEPES and CHES buffers

Super Buffer 2: composed of Malonic acid, Imidazole and Boric acid buffers

JBScreen Thermofluor SPECIFIC screens for high-scoring[(1)] mono-, di- and trivalent cations

pH		1	2	3	4	5	6	7	8	9	10	11	12
	A	CP + Dye CB-1	CP + Dye CB-1	CP + Dye CB-1	CP + Dye CB-2	CP + Dye CB-2	CP + Dye CB-2	CP + Dye CB-3	CP + Dye CB-3	CP + Dye CB-3	TP + Dye Original Buffer	TP + Dye Original Buffer	TP + Dye Original Buffer
	B	4.0	4.0	4.0 20 mM MgSO4	4.0 10 mM FeCl3	4.0 10 mM ZnCl2	4.0 10 mM MnCl2	4.0	4.0	4.0 20 mM MgSO4	4.0 20 mM CaCl2	4.0 10 mM ZnCl2	4.0 10 mM MnCl2
	C	5.0	5.0	5.0 20 mM MgSO4	5.0 10 mM FeCl3	5.0 10 mM ZnCl2	5.0 10 mM MnCl2	5.0	5.0	5.0 20 mM MgSO4	5.0 20 mM CaCl2	5.0 10 mM ZnCl2	5.0 10 mM MnCl2
	D	6.0	6.0	6.0 20 mM MgSO4	6.0 10 mM FeCl3	6.0 10 mM ZnCl2	6.0 10 mM MnCl2	6.0	6.0	6.0 20 mM MgSO4	6.0 20 mM CaCl2	6.0 10 mM ZnCl2	6.0 10 mM MnCl2
	E	7.0	7.0	7.0 20 mM MgSO4	7.0 10 mM FeCl3	7.0 20 mM LiCl	7.0 20 mM KCl	7.0	7.0	7.0 20 mM MgSO4	7.0 20 mM CaCl2	7.0 20 mM LiCl	7.0 20 mM KCl
	F	8.0	8.0	8.0 20 mM MgSO4	8.0 10 mM FeCl3	8.0 20 mM LiCl	8.0 20 mM KCl	8.0	8.0	8.0 20 mM MgSO4	8.0 20 mM CaCl2	8.0 20 mM LiCl	8.0 20 mM KCl
	G	9.0	9.0	9.0 20 mM MgSO4	9.0 10 mM FeCl3	9.0 20 mM LiCl	9.0 20 mM KCl	9.0	9.0	9.0 20 mM MgSO4	9.0 20 mM CaCl2	9.0 20 mM LiCl	9.0 20 mM KCl
	H	10.0	10.0	10.0 20 mM MgSO4	10.0 10 mM FeCl3	10.0 20 mM LiCl	10.0 20 mM KCl	10.0	10.0	10.0 20 mM MgSO4	10.0 20 mM CaCl2	10.0 20 mM LiCl	10.0 20 mM KCl

Columns 1–6: 100 mM Super Buffer 1; columns 2–6: 150 mM NaCl

Columns 7–12: 100 mM Super Buffer 2; columns 8–12: 150 mM NaCl

Super Buffer 1: composed of Citric acid, HEPES and CHES buffers

Super Buffer 2: composed of Malonic acid, Imidazole and Boric acid buffers

(1) Mostly occuring cations that appear as ligands in protein strucutres on the PDB (Protein Data Bank)