Anti-Freeze/Ice-Structuring Proteins

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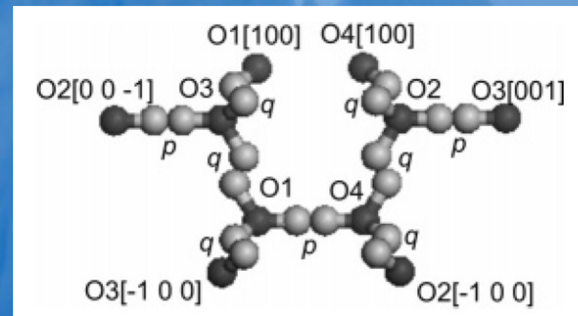
Bioinorganic Chemistry

Winter 2007

First things first: Structure of ice

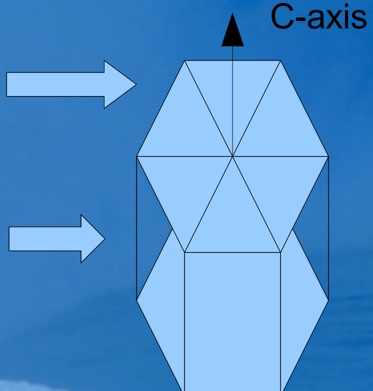
Under normal conditions, ice has a hexagonal lattice structure.

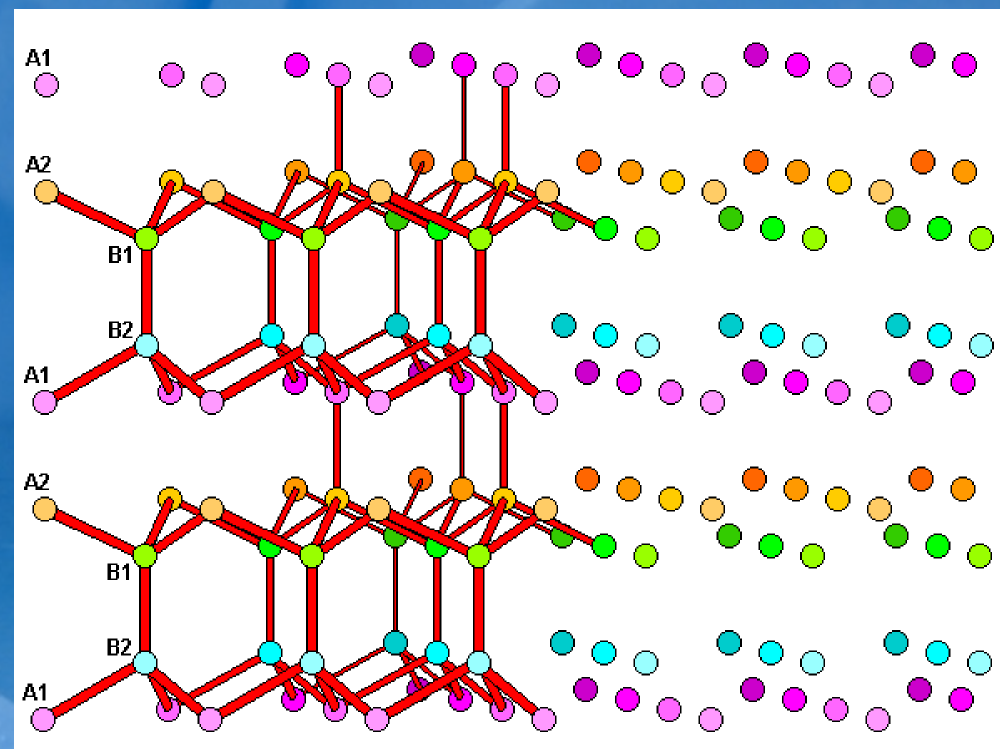


Unit cell of hexagonal ice.
Strom 2005

Basal Plane

Prism Plane





http://www.uwgb.edu/dutchs/PETROLGY/Ice%20Structure.HTM

Macroscopic Signs of AFP activity

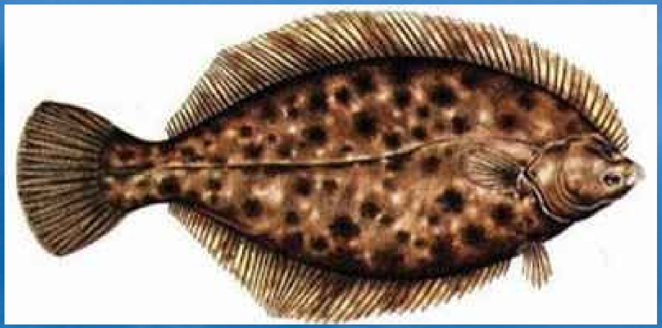
Tenebrio Molitor
(Mealworm Beetle)
TH: 1.4°C at 20uM

http://www.the-piedpiper.co.uk/th7h.htm

Winter Rye
TH: not much, but crystal size small
http://homepage.mac.com/cohora/con/Spring.html

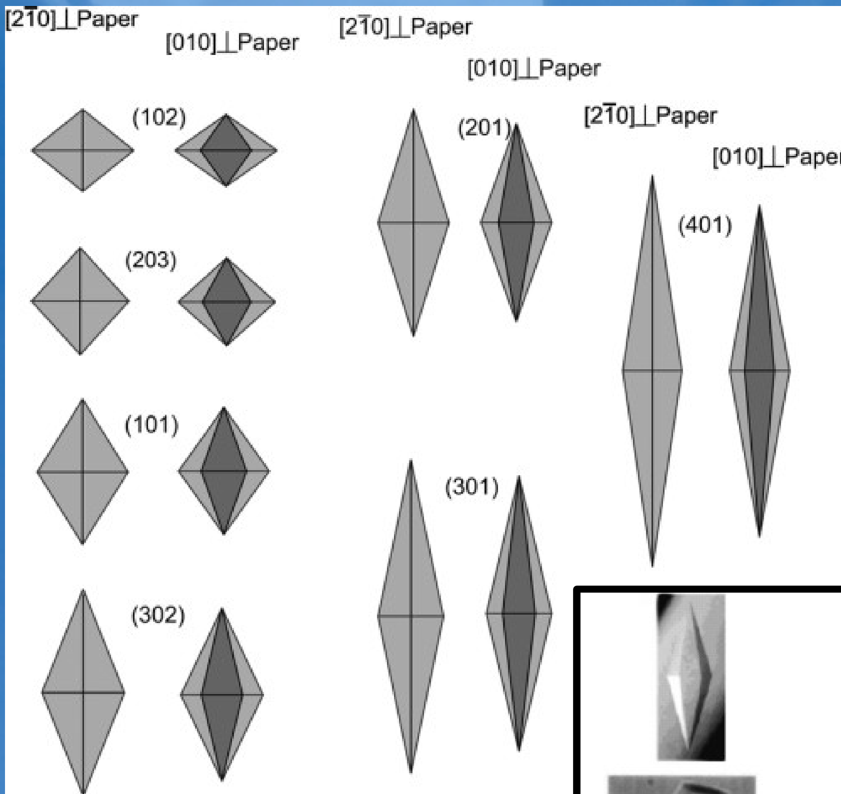
For comparison:
f.p. depression of NaCl:
1.4°C at 0.376 M

Ocean Pout (type III)
TH: 1.4°C at 2.8mM
http://www.afprotein.com/pout.htm

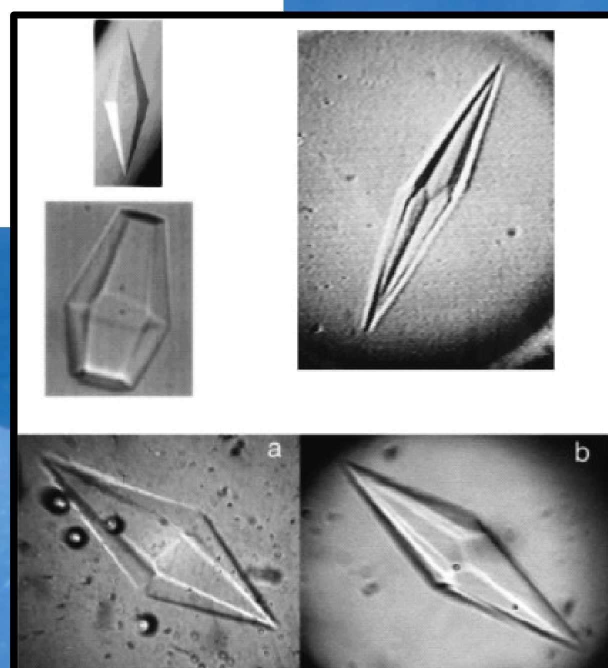


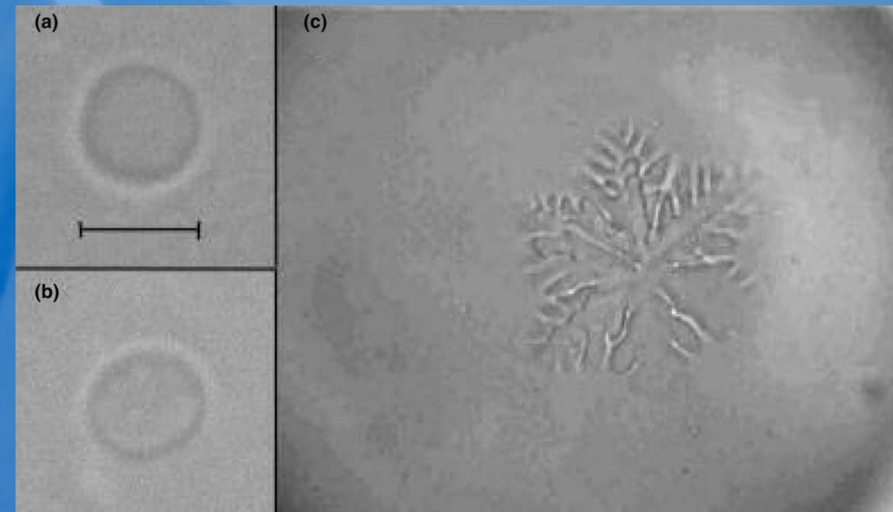
Winter Flounder (type I)
TH: 0.27°C at 400uM
http://www.cptdave.com/winter-flounder.html

Microscopic Signs of AFP activity



Morphologies obtained from different antifreeze protein solutions
Strom 2005





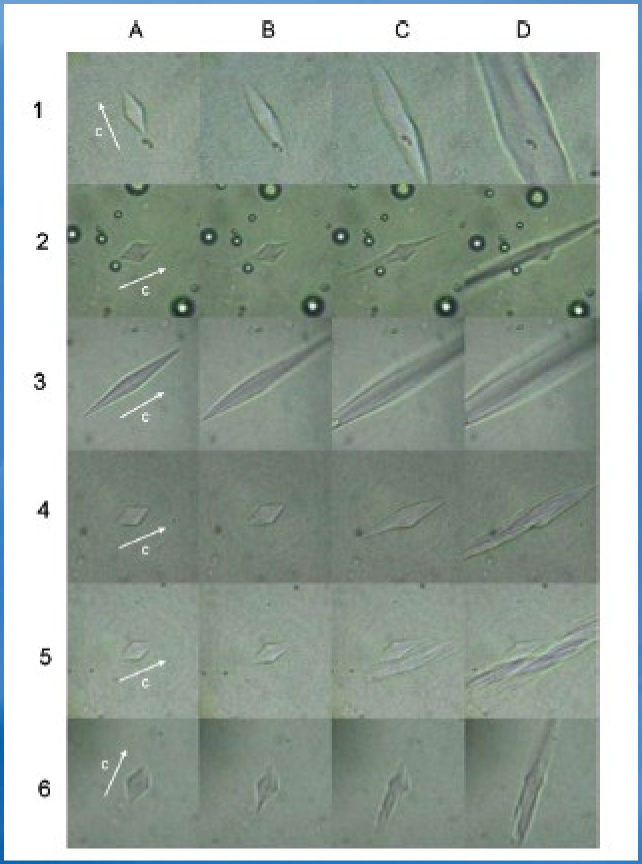
Left: two crystals at 0.3 °C undercooling. Right: burst pattern below hysteresis f.p.
Gilbert 2005

Thermal Hysteresis

Freezing point depression – minus the accompanying change in melting point

Specifically, the difference in temperature between the freezing and melting points of ice

Solutions of proteins with embryonic ice crystals show no growth, but beyond hysteresis point – explosive growth!



Ice growth in various AFP solutions, 0.4 s intervals between images. *Scotter 2006*

Mechanisms/explanations coming right up!

Ice-structuring proteins – Two different ways to function

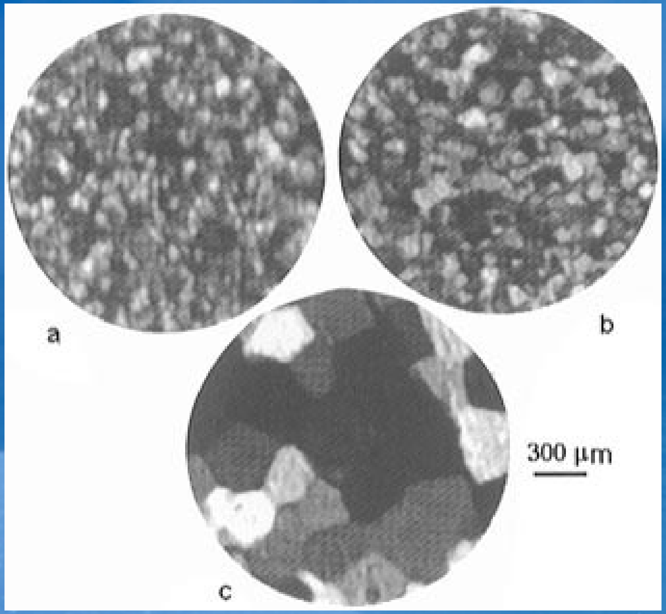
Ice Nucleation – formation of initial seed crystals, if no other nucleation sites exist

-experiments have shown AFP's to be mediocre at best

Recrystallization – coalescence of small ice crystals into larger crystals due to minimization of the overall grain boundary energy

-Food: freezer burn, freeze ripening, bad ice cream

-Biology: cell death



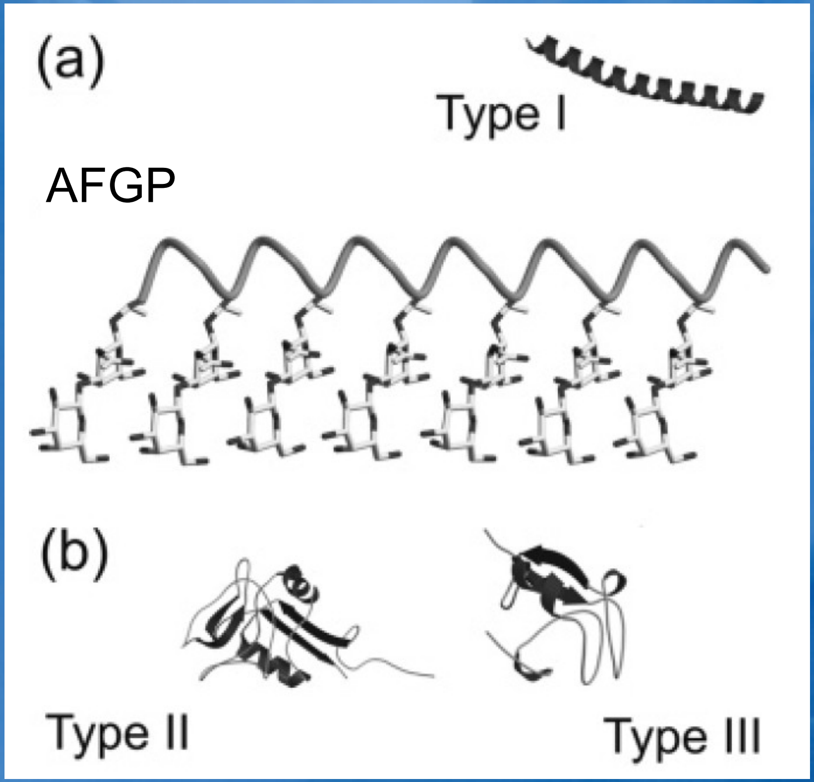
Grain sizes of various solutions – top row: AFGP 4 at 0.1 ug/mL, AFGP 8 at 0.5 ug/mL. Bottom: pure water *Yeh 1994*

Overcoming Crystal Formation/Growth

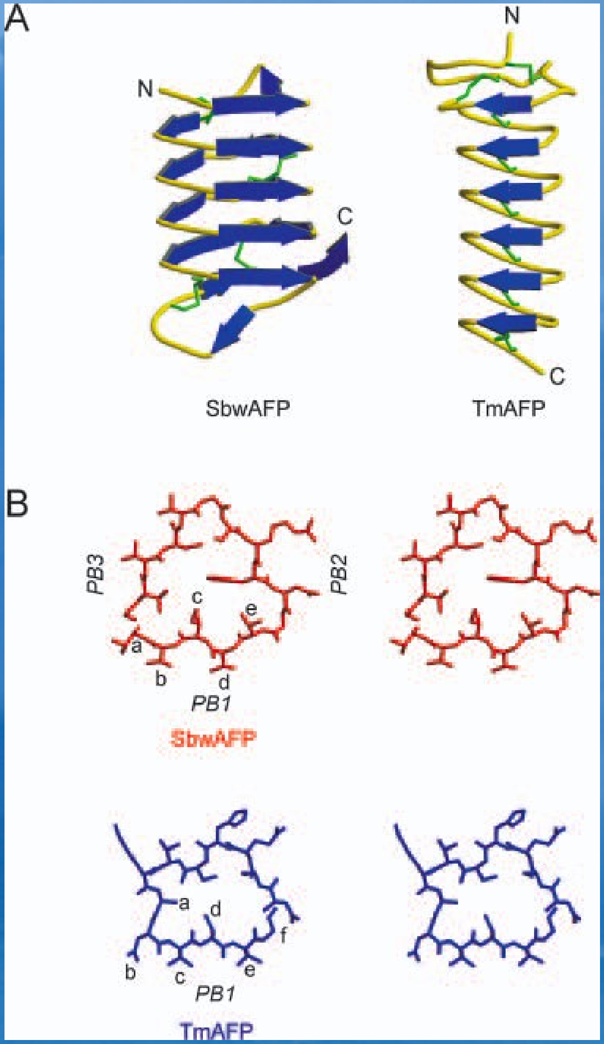
Possible solutions:

- -Colligative properties (not necessarily viable)
  - -lower freezing/melting point of liquid
  - -Danger: high osmotic pressure
- -Non-colligative based approaches
  - -prevent recrystallization structurally
  - -lowers freezing point, but not melting point
    (Thermal hysteresis)
- -Not mutually exclusive – these can work simultaneously
  (Glycerol in rainbow smelt, Driedzic 2004)

Incredible structural diversity



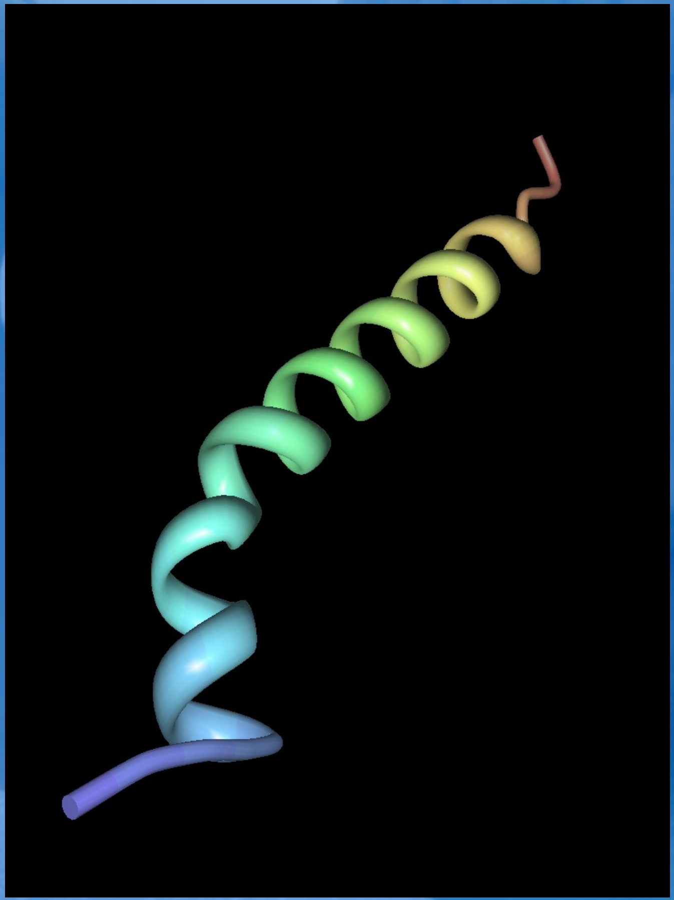
Fish AFP's
Strom 2005

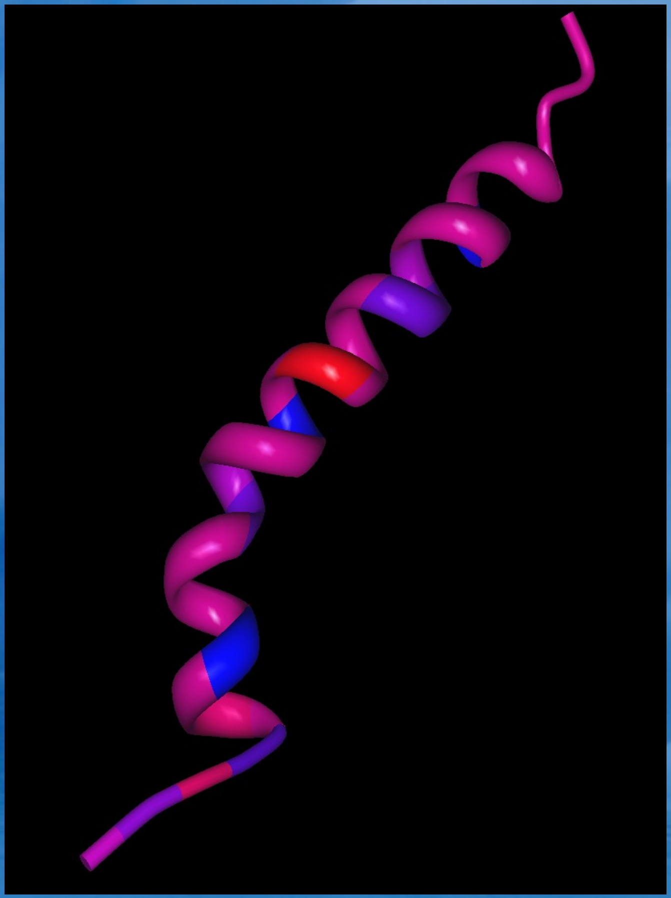


Insect AFP's
Graether 2004

Type I AFP's – Winter flounder

Chain color ramp





Hydrophobicity (red = most blue = least)

Structure: *Kwan 2005*

Type I AFP's – Winter flounder

-Characterized by 3-4 kDa lone α-helices, 11-residue repeat unit: $ThrAla_2AsxAla_7$ (Asx = either Asp or Asn)

-Heavy in alanine composition

-Rotamer preference: winter flounder AFP has 4 Thr residues, and the middle two have a preference for -60 degrees 55% of the time

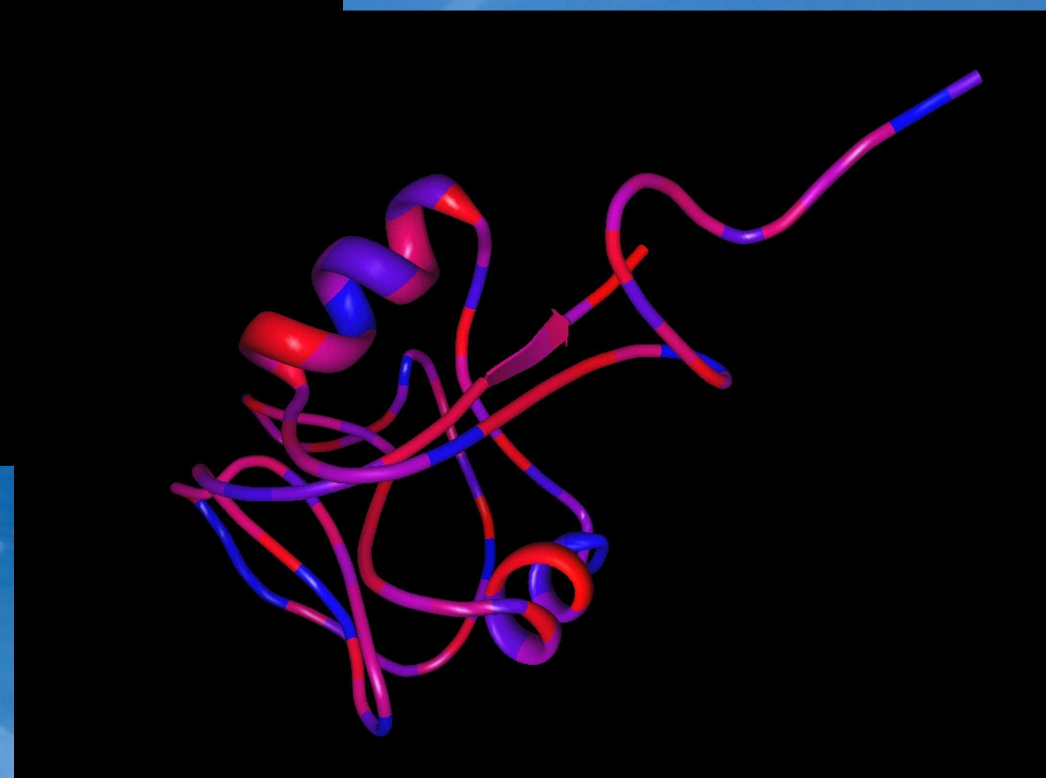
-Simple, easy to synthesize. Hence, easy to study.

- -Synthesis tests on hydrophobicity vs. size/pattern matching

Note some “hyperactivity” possible for longer α-helices (Inglis 2006, Marshall 2005)

Type II – Sea Raven, smelt, herring

Structure: *Gronwald 1998*



Type II – Sea Raven, smelt, herring

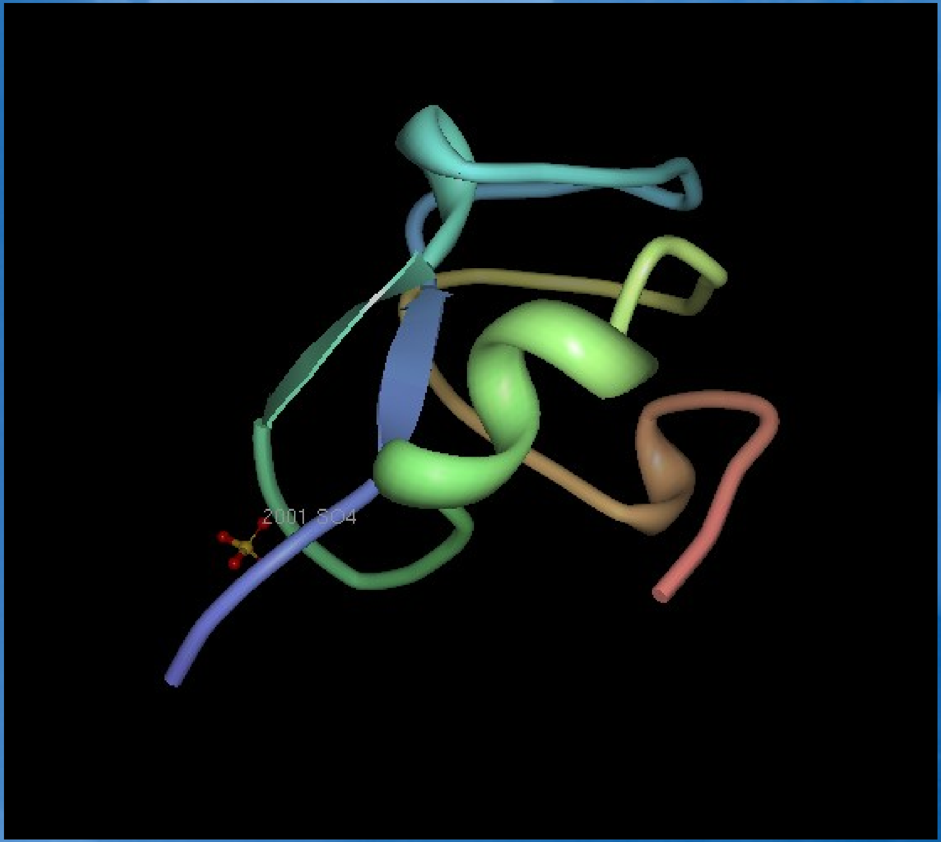
Characterized by 11-24 kDa proteins with globular lectin fold, homologous to carbohydrate binding domain of C-type lectins

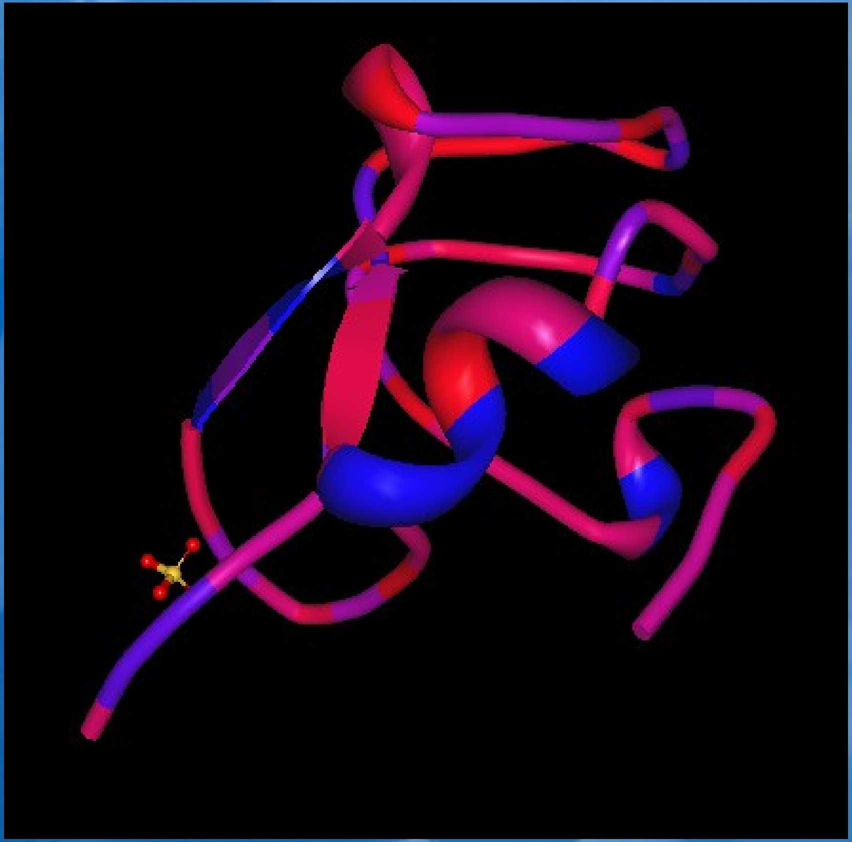
Cysteine-rich

Interesting evolutionary chemistry – herring AFP II requires Ca^{2+} as a cofactor, but sea raven AFP does not.

The mightiest tree in the forest might be easier to cut down if herring didn't have AFP...

Type III – ocean pout, wolfish, eel pout





Structure: *Antson 2001*

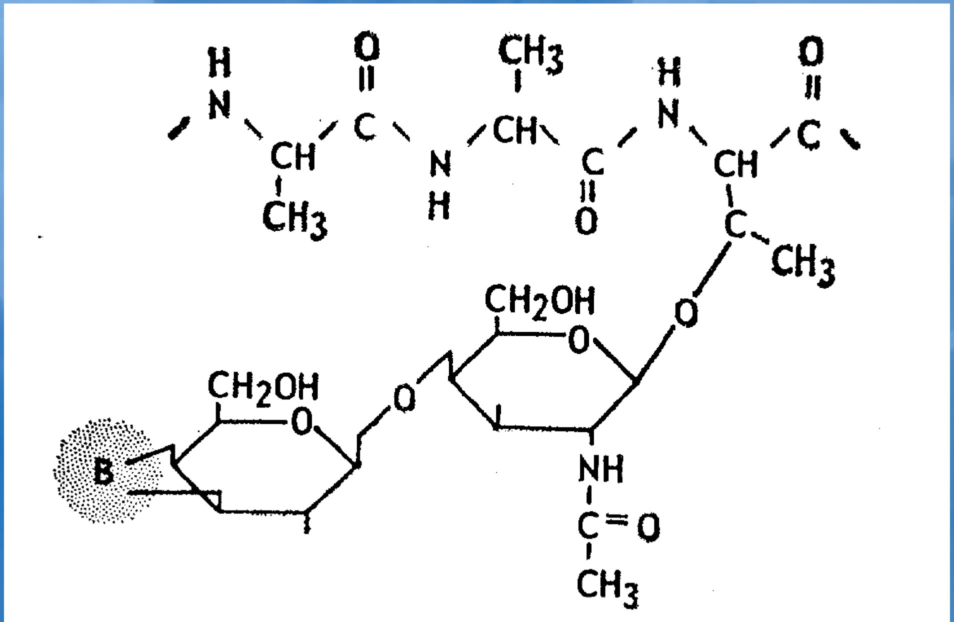
Type III – ocean pout, wolfish, eel pout

Main characteristic: 6.5 kDa β-clip fold/sandwich

62% apolar surface!
(binding face is even more hydrophobic, but has some polar regions)

Aggregation plays important role in activity – gathering on an ice nucleation site promotes optimal packing of very hydrophobic AFP molecules, allowing minimization of net hydrophobic area

Antifreeze glycoproteins



Devries 1971

Main Characteristic: polymeric tripeptide (Ala-Ala-Thr) units with attached disaccharide residues (3-*O*-(*α*-D-galactosyl)-D-*N*-acetylgalactosamine)

-no long-range order, numerous conformers in soln

Many distinct fractions – from 4-50 repeat tripeptide units, with activity increasing with length.

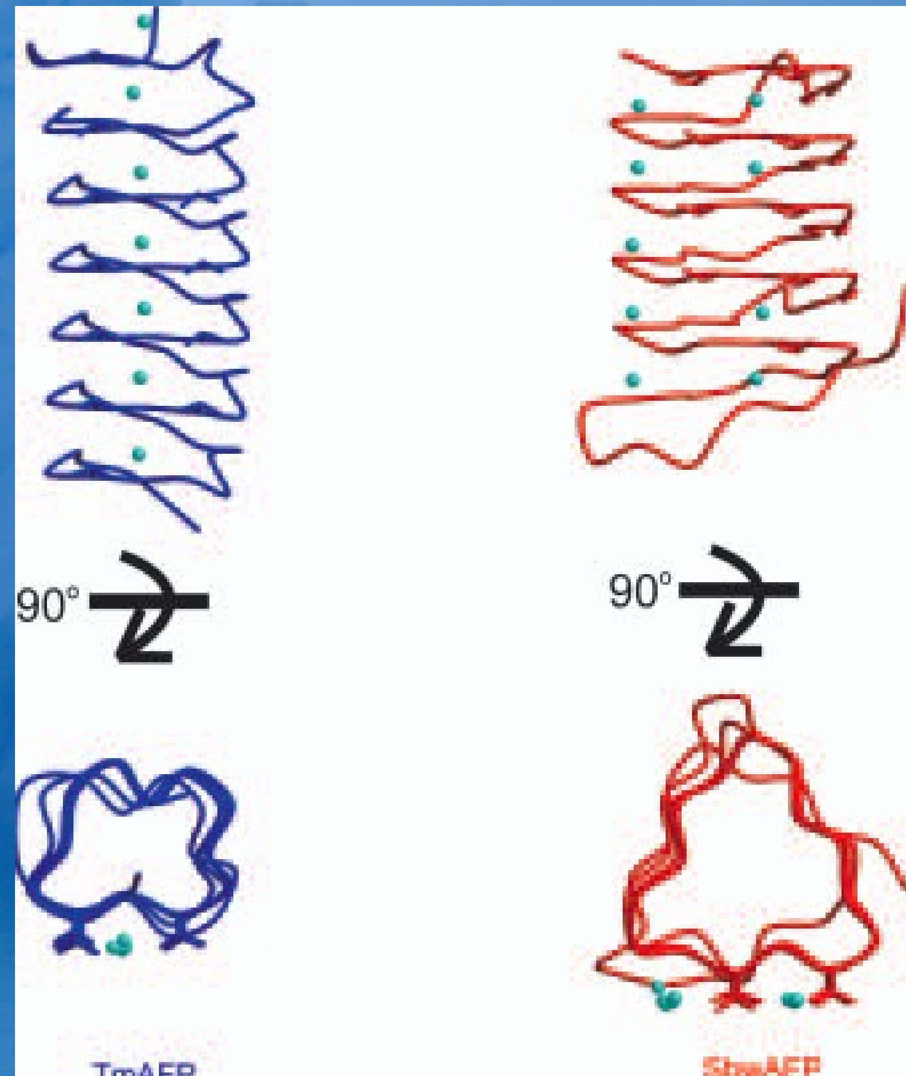
Cooperative behavior – smaller glycoproteins have enhanced activity in presence of larger glycoproteins (not observed with AFP's)

Insect AFP's

Typically much higher activity than fish AFP - “hyperactive”

β-helical structure

2-dimensional binding



Insect AFP's from T. Molitor and the spruce bud worm
Graether 2004

Mechanism for activity

Important Details:

Binding: hydrogen bonding or van der Waals interactions?

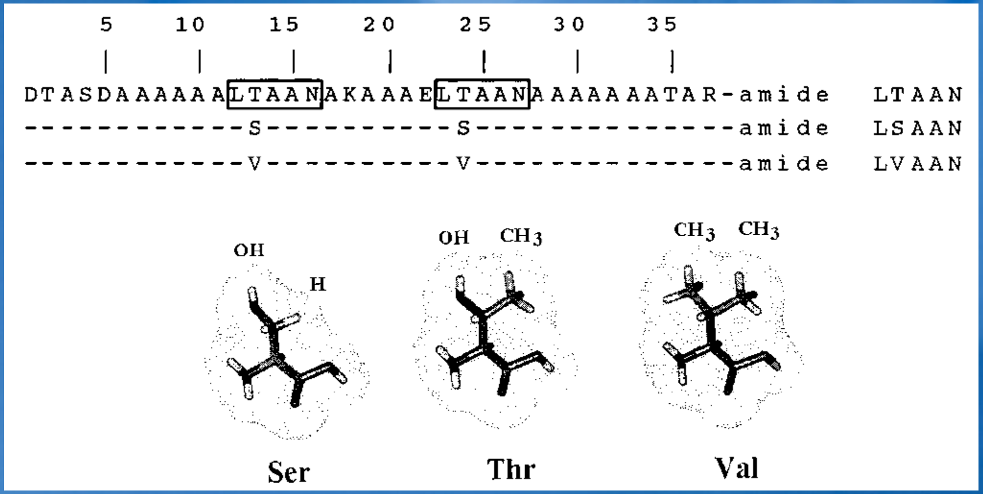
Reversible or irreversible binding?

Which face(s)?

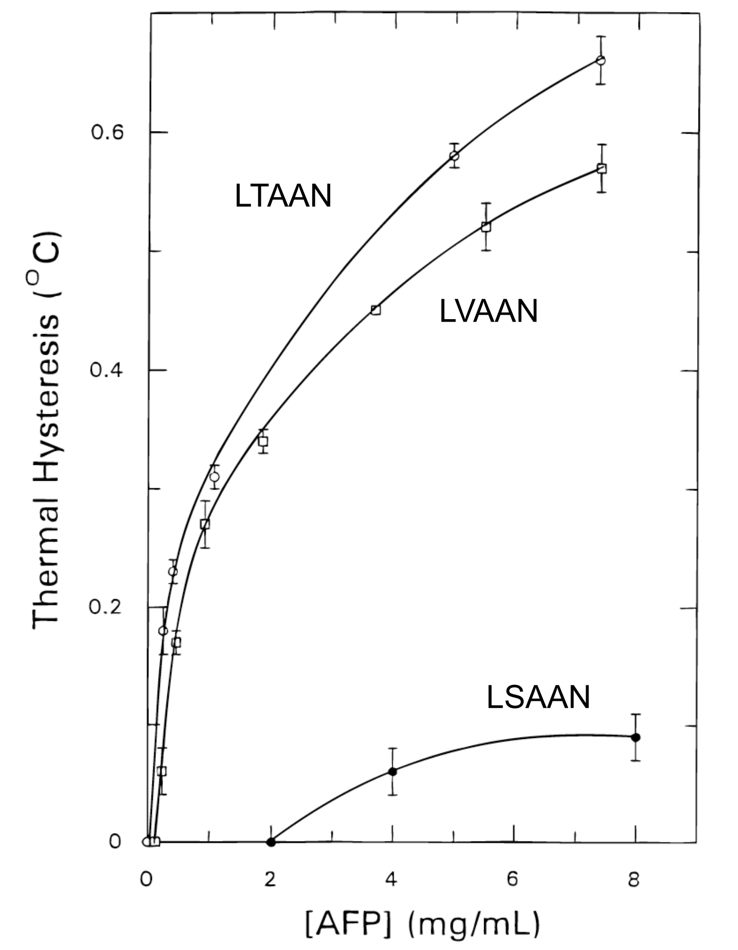
Mechanism for activity

Hydrogen bonding or van der Waals – how to tell?

mutagenesis/synthesis of mutants!



Type 1 mutant AFP synthesis & results
Chao 1997



Mechanism for activity

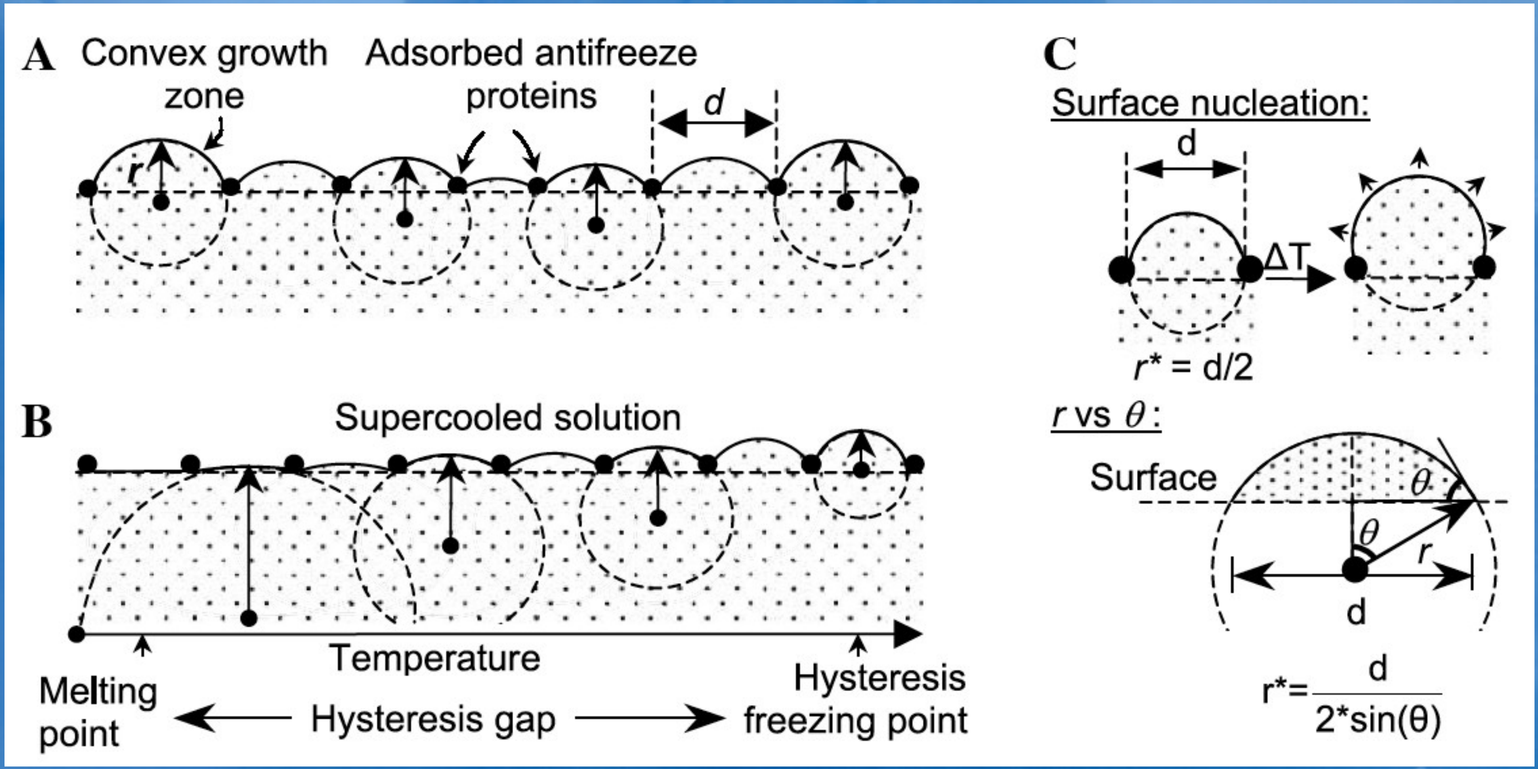


Illustration of Kelvin theory of thermal hysteresis
Kristiansen 2005

Mechanism for activity

Kelvin effect:

$$\Delta T = \frac{2 \Omega \gamma T_o}{\rho_{min} \Delta H_o}$$

Yeh, 1996

Ω = Ice molar volume
γ = Isotropic surface energy
ρ = ice particle sphere radius
T_0 = Original freezing point of water
ΔH_0 = molar heat of fusion for water

Depression is due to convex growth zones – increasing surface area.

Smaller gaps between proteins -> smaller growth zones -> larger surface area -> more freezing point depression

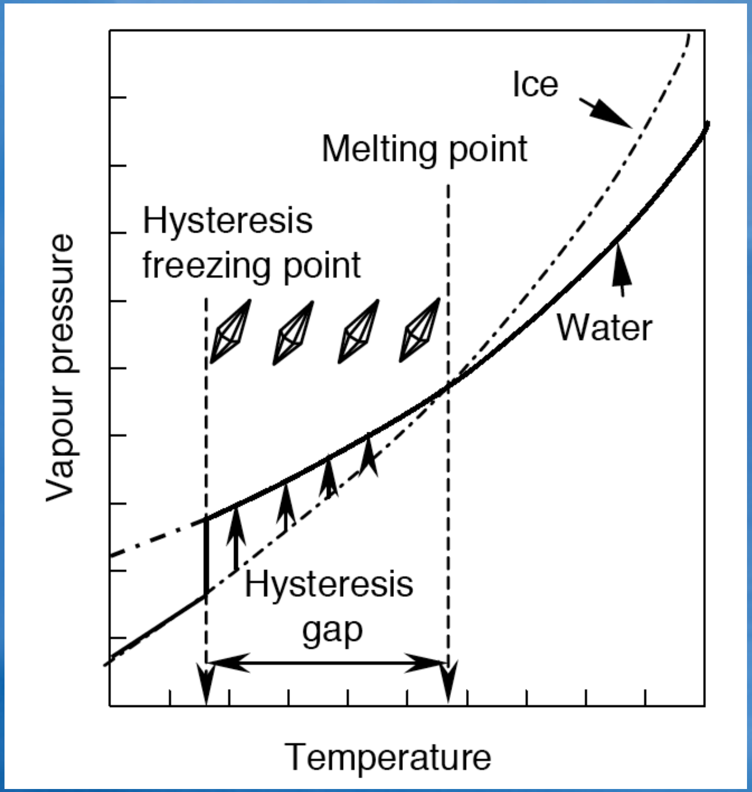
Alternate proposals

Pressure explanation

Capillary action: model for understanding

convex curvature increases the vapor pressure below the surface, lowering the liquid level in a capillary tube

Concave curvature decreases the vapor pressure below the surface, raising the liquid level in a capillary tube.



Kristiansen 2005

Hence, by forming convex surfaces on the embryonic ice crystal or ice nucleating agent, the ice vapor pressure is increased to the vapor pressure of water, maintaining equilibrium and preventing the crystal from growing.

Mechanism for activity

Reversible or irreversible binding?

- Irreversible binding: a good starting point
  - Theoretically simple, explains behavior
    - –proteins get in the way, ice grows between them, ice eventually reaches thermodynamic equilibrium due to its surface area or vapor pressure

- Why that's not the whole story:
  - -bond strength necessary for irreversible adsorption
  - -absence of a clearly defined surface to which the antifreeze proteins may adsorb.

Alternate proposals

Reversible binding – because permanence was too easy.

$$\Delta T = \frac{4\Omega\gamma T_0}{\Delta H_0\left[\sqrt{Ne} - \frac{2b}{\Pi}\right]}$$

Yeh, 1996

Ne = number of adsorbed protein molecules, according to Langmuir adsorption theory

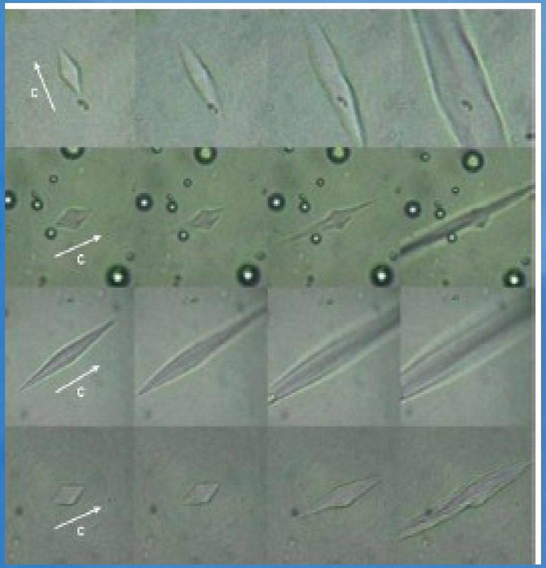
Mechanism for activity

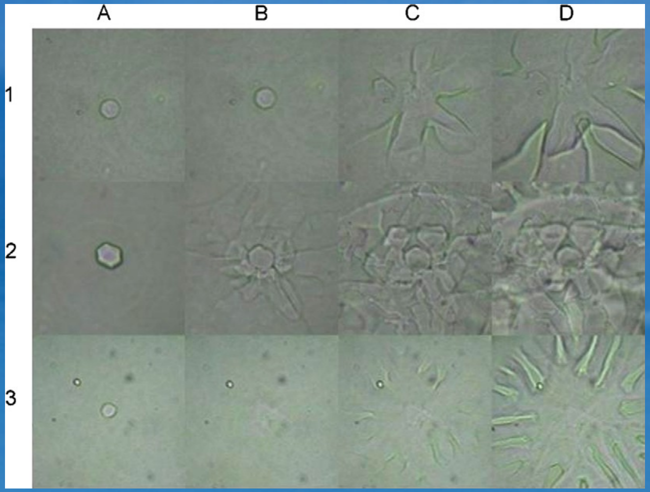
Which face?

- -Depends on the protein
- -How to tell – crystal shape beyond Hysteresis freezing point “explosive growth”.

Faces that don't expand are bound.

- -Insect, hyperactive AFP's bind basal planes, form snowflake patterns
  - -Higher activity (10-100 fold)
- -Fish AFP's bind prism planes, form spicules





Top: fish AFP beyond TH f.p.
Bottom: insect AFP beyond TH f.p.
Scotter, 2006

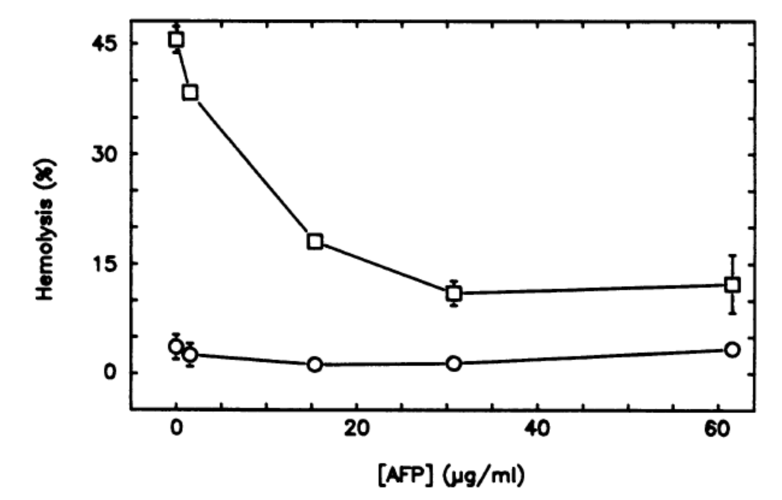
Applications in Society

Enhanced storage times for blood, less damage after freezing

AFP Type	Source of AFP	Cell type	AFP conc	Key results	Other Observations:
I	Recomb. Identical to winter flounder	Red Blood Cells	0 ug/mL	45% hemolysis after rapid freeze/slow re-warm	Near negligible hemolysis after rapid freeze/rapid rewarm
			0-150 ug/mL	12% hemolysis after rapid freeze/slow rewarm	Higher concentrations led to increased hemolysis
II	Hemitripterus Americanis		~30uM	Similar	
III	Recombinant		~30uM	Similar	

Re-created from Inglis, 2006

Plot of red blood cell hemolysis vs. AFP concentration.
Circles: fast warm in water bath.
Squares: slow warm in air.
Carpenter, 1992



Applications in Society

Cryopreservation of mammalian embryos and oocytes

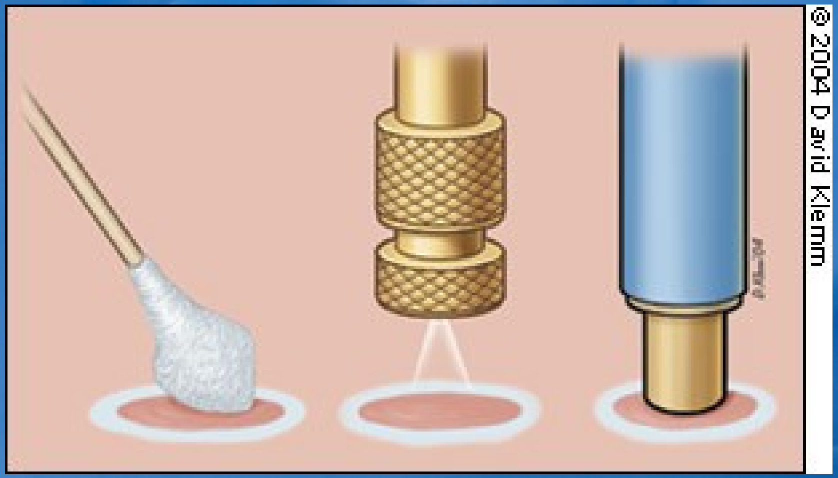
http://www.advancedfertility.com/freezer.htm

AFP type	Source	System	Concent.	Results
I	Winter Flounder	Bovine oocytes	20mg/mL	4-fold increase of oocytes retaining intact oolemma after treatment at 4 C for 24 h; 3-fold increase of in vitro fertilization rates
I	Winter Flounder	Human oocytes	1mg/mL	90% monospermic fertilization after treatment at 4 C for 20 h

Reconstructed from Inglis 2006

Applications in Society

Cryosurgery



Liquid nitrogen applicators used in cryotherapy
http://www.aafp.org/afp/20040515/2365.html

Cryosurgery or cryotherapy is the destruction of unwanted tissue by freezing it, generally with liquid nitrogen.

Useful for both external and internal cancers – skin, liver, cervical,bone – mostly effective against early developmental stages.

Fewer side effects, shorter recovery times than other cancer treatments

Perhaps AFP's could be used to protect healthy tissue from injury.

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21)http://www.cancer.gov/cancertopics/factsheet/Therapy/cryosurgery

22)http://sprott.physics.wisc.edu/fractals/collect/2004/ - fractal background for front slide

23)http://studyabroad.msu.edu/photocontest/05_winners/honor_mentions/pages/ANTARCTICA%20-%20Iceberg%20Lagoor - background for all other slides