# Криоактивные вещества

# полипептидной природы

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## ***PROTEIN SEQUENCE – STRUCTURE - FUNCTION***

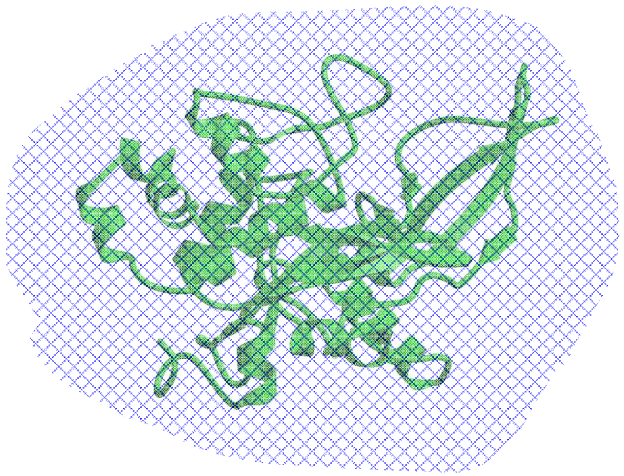
**Principal activities:**

Bioinformatics analysis of protein sequences and structures

Structural prediction and molecular modelling

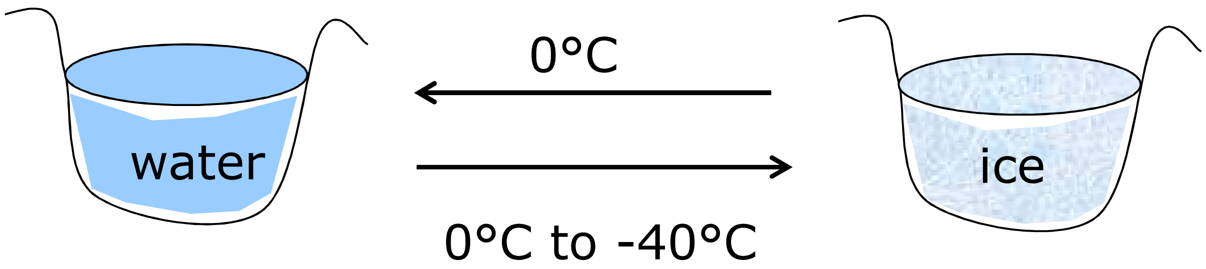
Molecular design

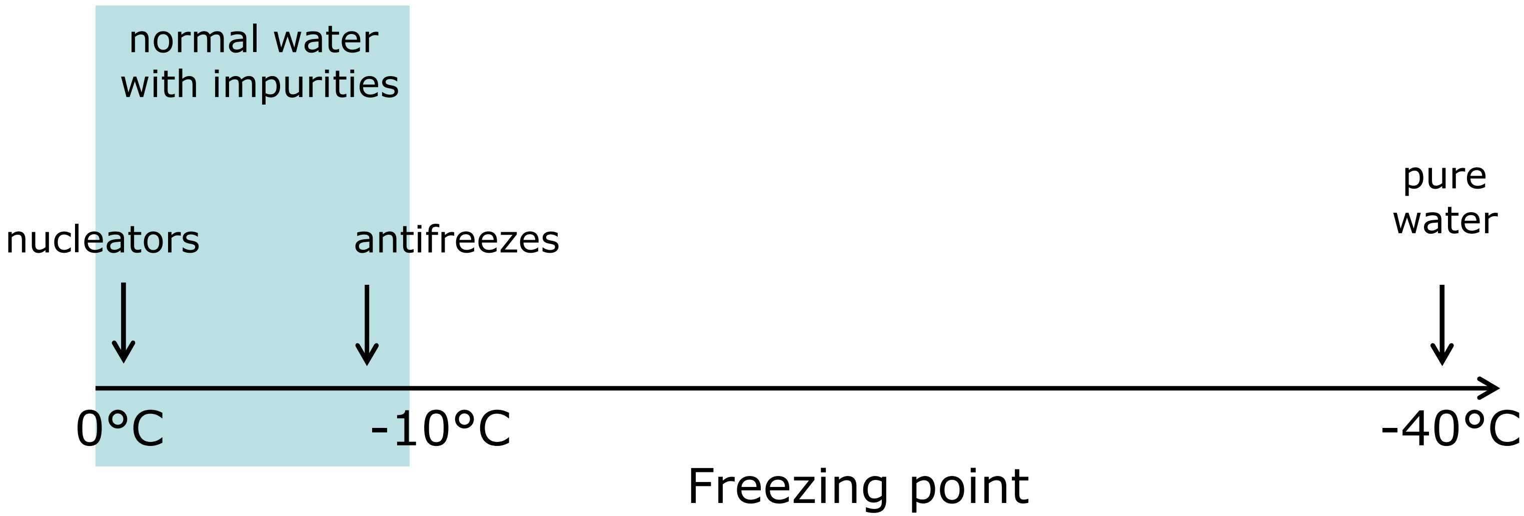
# Water and protein structures affect each other



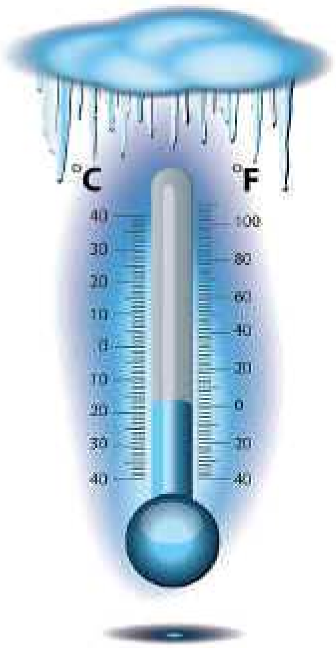
Melting point of ice is 0 °C.

Freezing point of water varies from 0°C to −40 °C depending on the presence of impurities



# Applications of ice-active molecules




Agriculture (thermosensitive plants, etc)

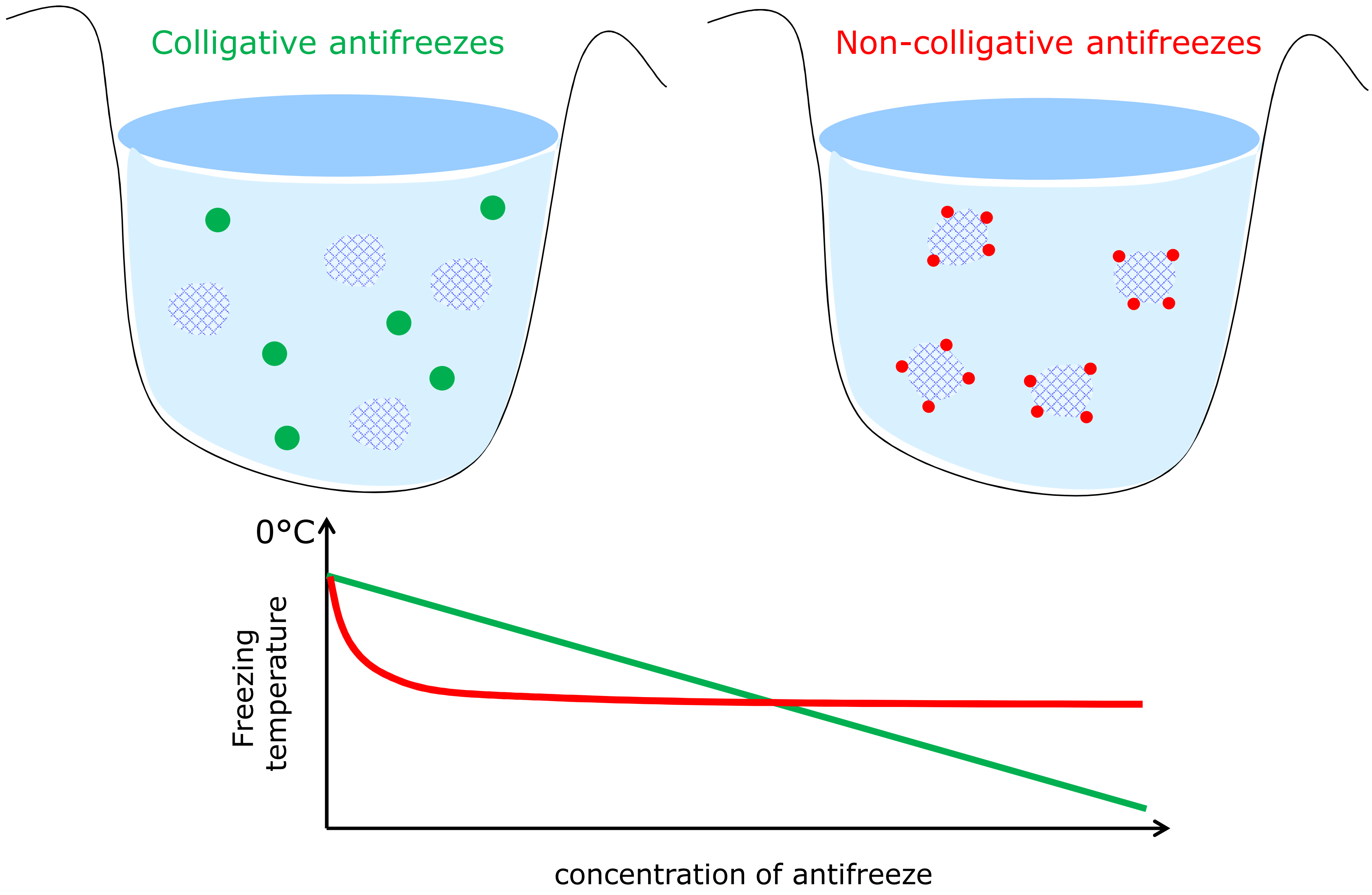
Climate, ecology (troposphere)

Food industry

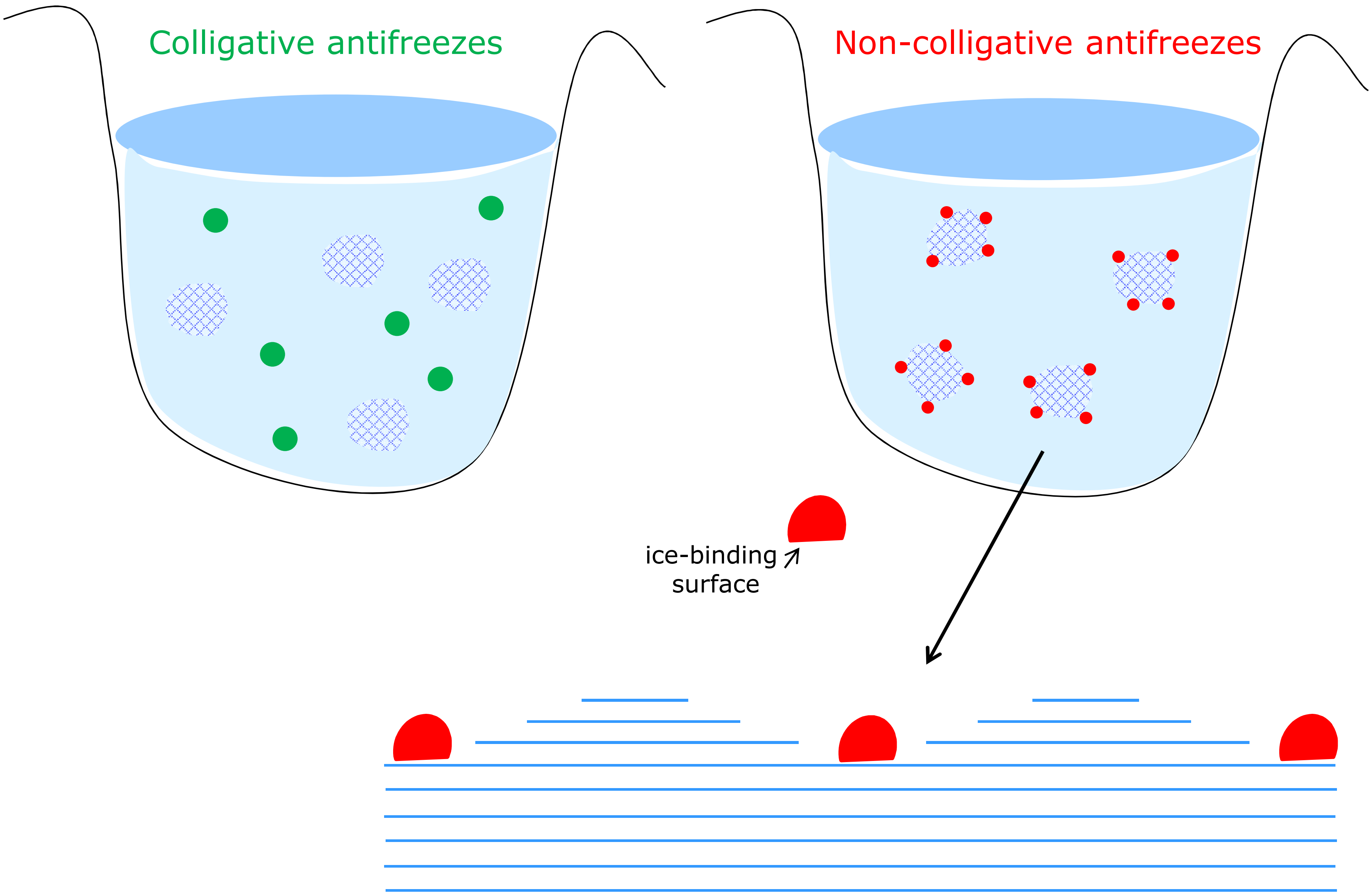
Medicine (conservation of cells (blood, sperm), organes and organisms)

Saving of energy

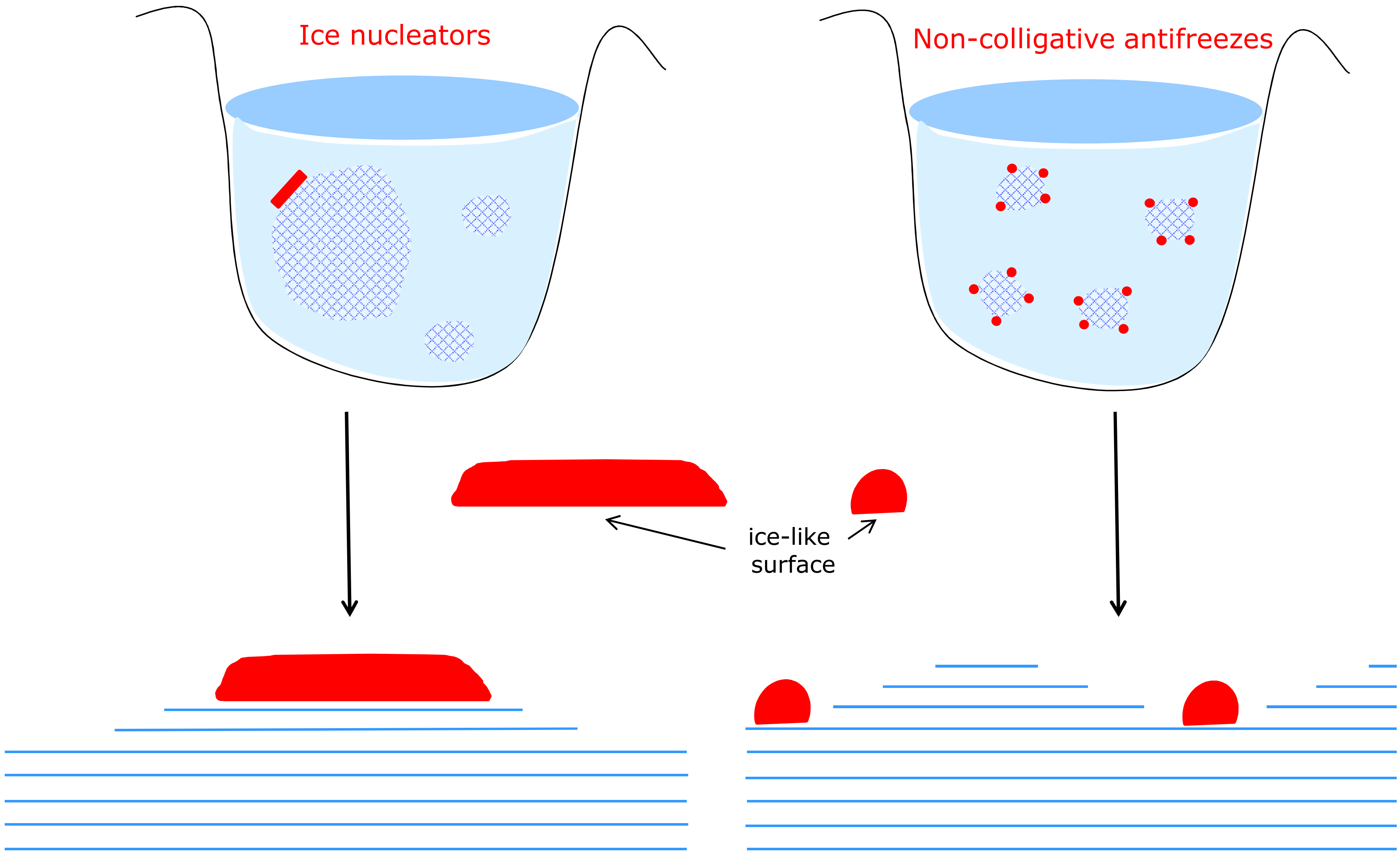
# Antifreezes



# Antifreezes



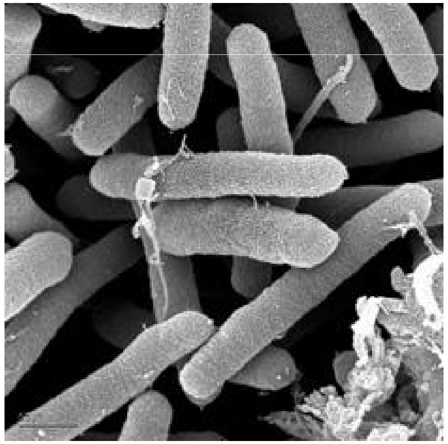
# Antifreezes vs Ice Nucleators



# Biological ice-active molecules


**Colligative antifreezes**
(glycerol, sorbitol)

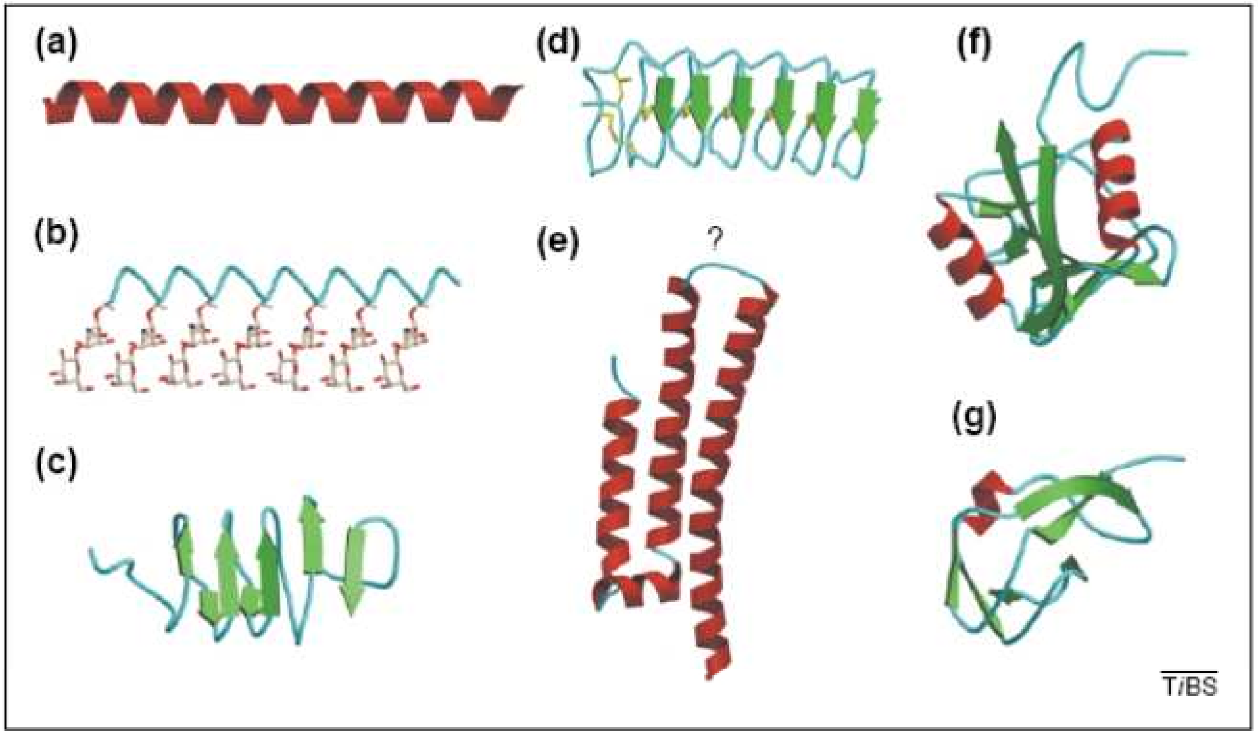
**Non-colligative antifreezes**
(peptides, proteins)

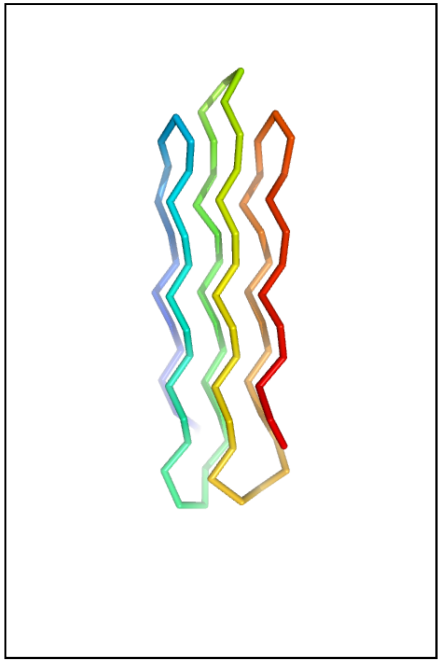


**Ice-nucleators**
(proteins)

Billions years of evolution
Non-toxic

# Known 3D structures of AFPs



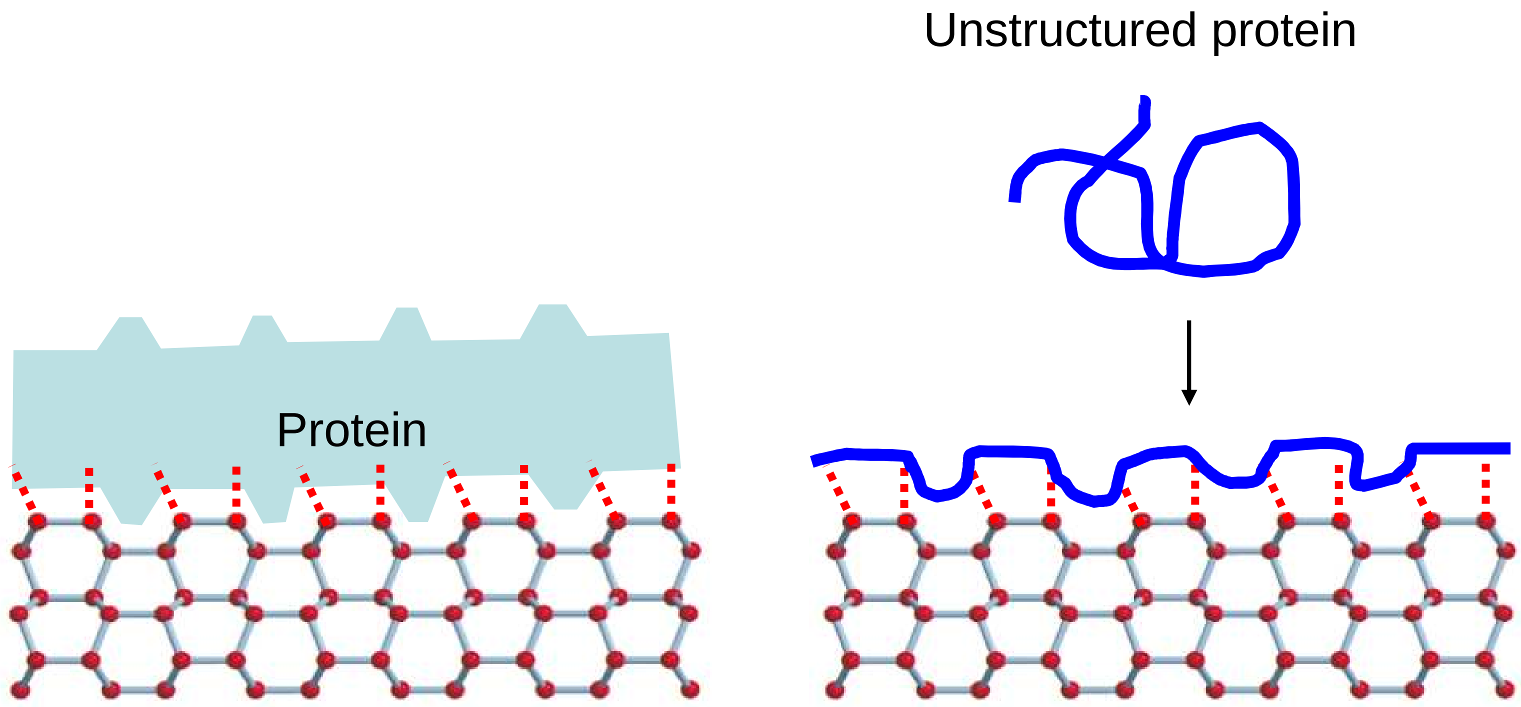
**Snow flea AFP** Pentelute et al. (2008)
J.Am.Chem.Soc. 130: 9695-9701

**Table 1. Antifreeze proteins from fish and insects[a]**

| Type | Classification | Size (kDa) | Repeat |
|---|---|---|---|
| Fish | | | |
| I | Alanine-rich α helix | 3–5 | 11aa (~3 turns of helix) |
| II | C-type lectin fold of mixed α, β and loop structure | 14–24 | None |
| III | Globular protein contains short β strands | 7 | None (but natural 14-kDa dimer observed) |
| IV | Helix-bundle (predicted) | 12 | 22 aa? |
| AFGP | Antifreeze glycoprotein | 3–24 | 3 aa (Ala–Ala–Thr)<br>\|<br>disaccharide |
| Insects | | | |
| Tm and Dc | Right-handed β helix | 8–9 | 12–13 aa (containing Thr–Cys–Thr) |
| Cf | Left-handed β helix | 9–12 | 15 aa (containing Thr–Xaa–Thr) |

[a]Abbreviations: AFGP, antifreeze glycoprotein; Cf, *Choristoneura fumiferana*; Dc, *Dendroides canadensis*; Tm, *Tenebrio molitor*; Xaa, any amino acid.

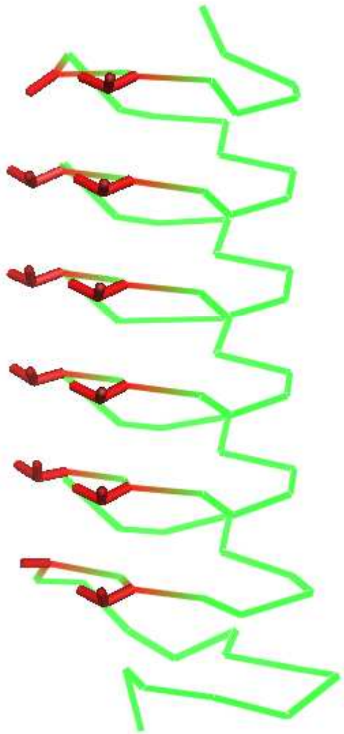
# Ice-like surface of AFPs

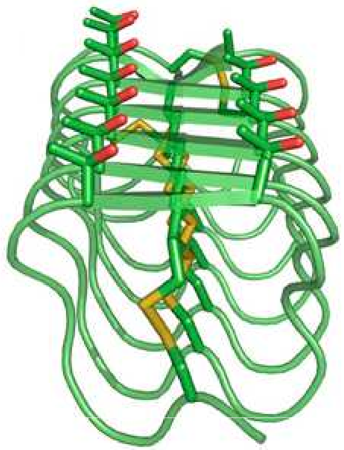


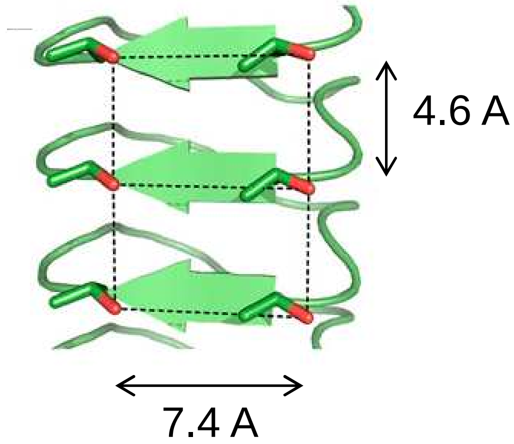
# Ice-like surfaces of AFPs

**Antifreeze protein from *Tenebrio molitor***

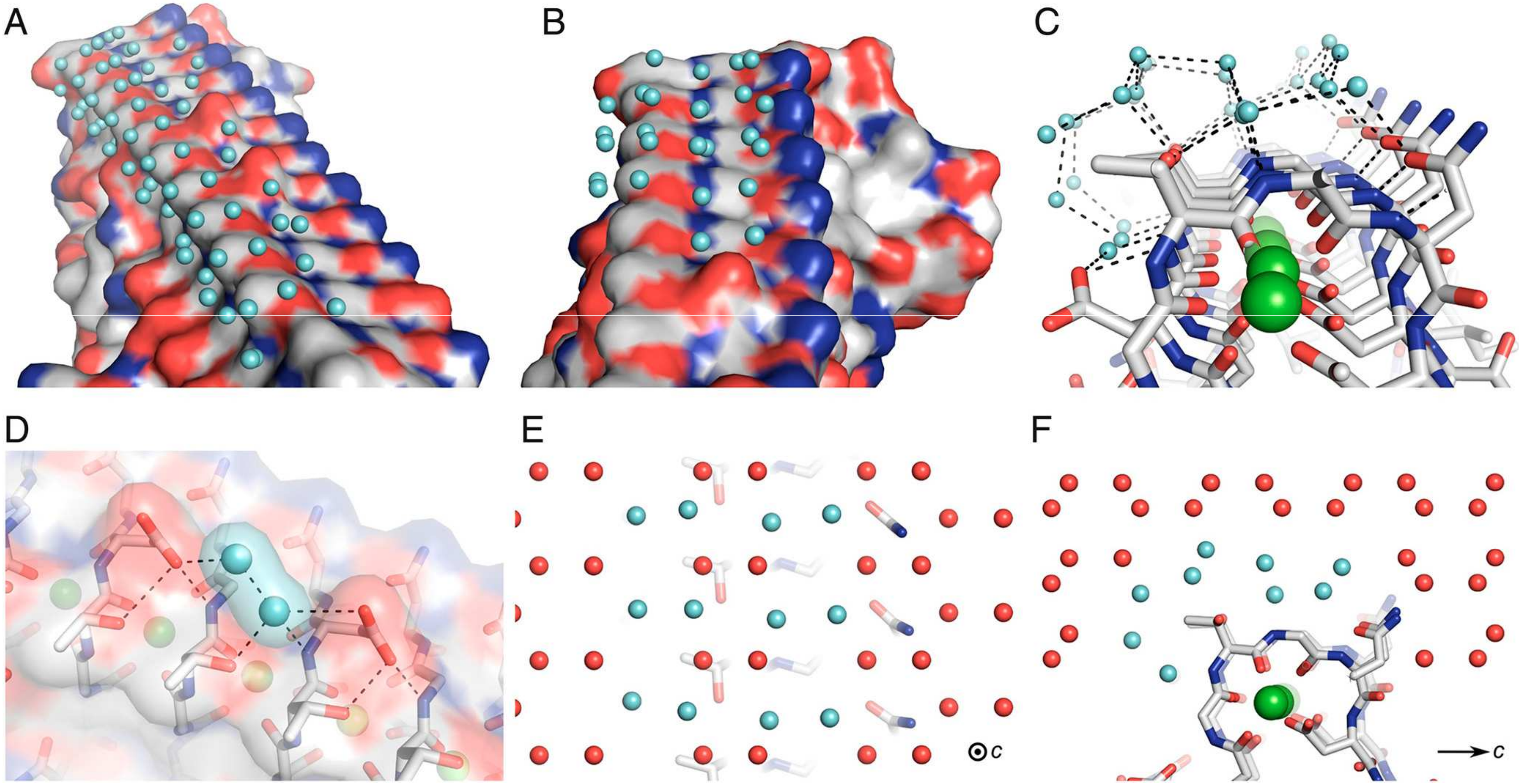
Q**C**TGGAD**C**TSCTG
A**CT**GCGN**C**PN**A**V
**TCT**N**S**QH**C**VK**A**N
**TCT**G**S**TD**C**NT**A**Q
**TCT**N**S**KD**C**FE**A**N
**TCT**D**S**TN**C**YK**A**T
A**CT**N**S**SG**C**P





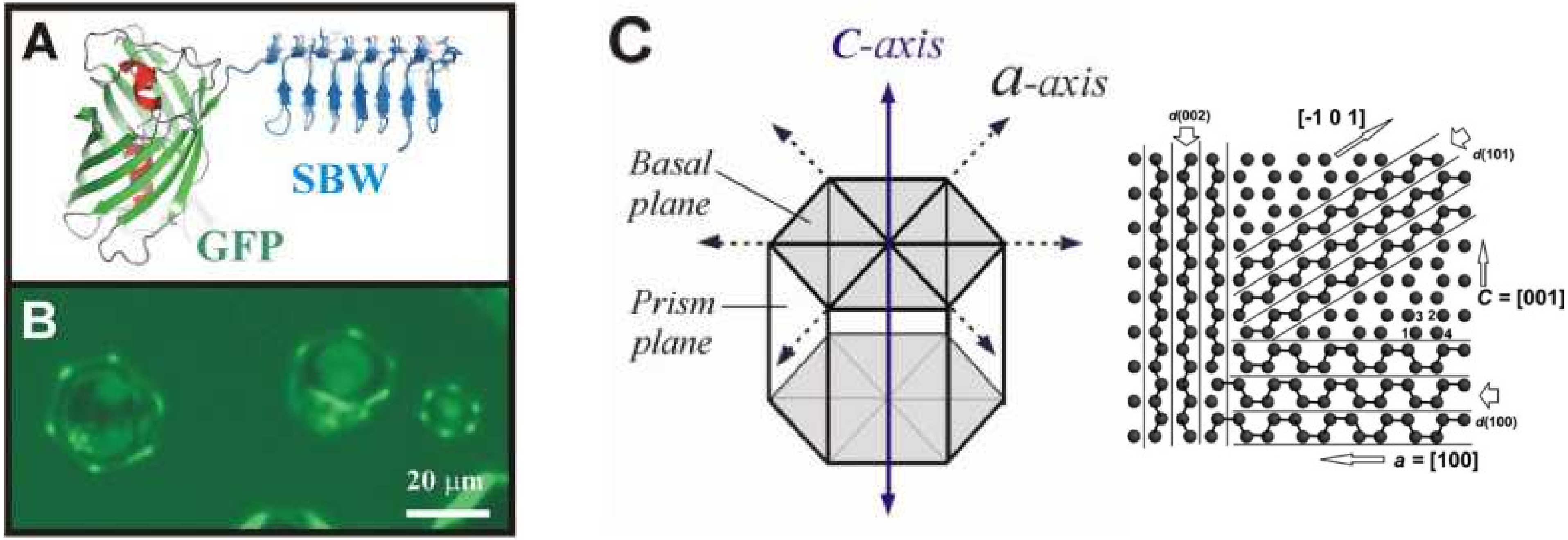


# Ice-like surfaces of AFPs




Garnham et al. *PNAS May 3, 2011 vol. 108 no. 18 7363-7367*

## Ice-like surfaces of AFPs




Pertaya et al. J. Phys.: Condens. Matter **19** No 41 2007 412101 (12pp)

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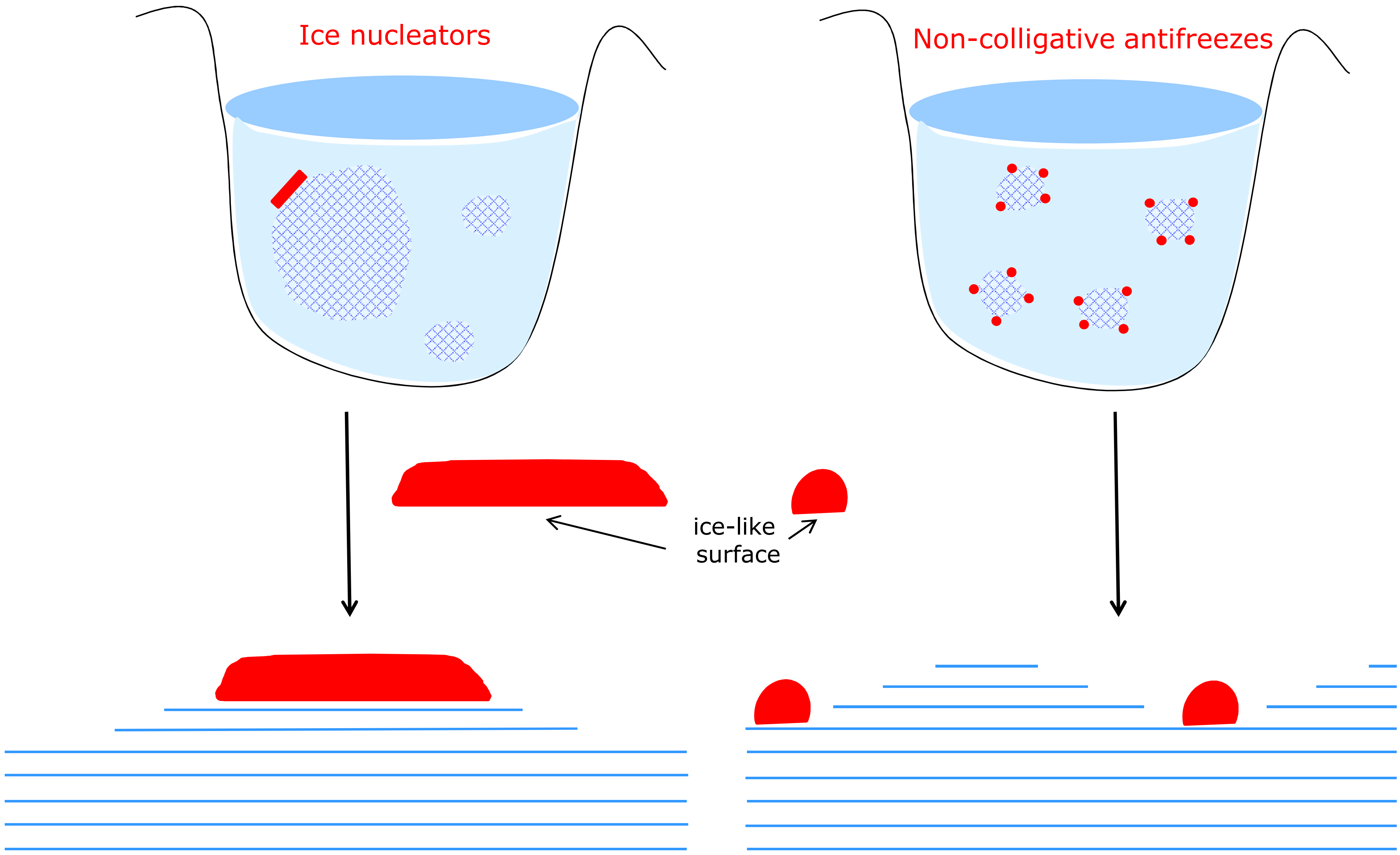
# Antifreezes vs Ice Nucleators



# BACTERIAL ICE-NUCLEATION PROTEINS

# Freeze toll to crops hits $670 million

ASSOCIATED PRESS

FRESNO — Damage to California's crops from the pre-Christmas freeze reached $670 million in reports from major farming counties compiled Wednesday.

Boards of supervisors in areas hard-hit when temperatures plummeted to the low 20s and high


Professor Steve Lindow

Department of Plant and
Microbial Biology
University of California,
Berkeley

« Intensively self-motivated, Steve (*Lindow*) rarely took time off work for more than classes, food, and, little sleep. On rare occasion (*in 1975*), faced with choice of working or a weekend of skiing, he put the active fractions (*of leaves*) in the coldroom and went skiing. When Steve returned on Monday, the extract had become turbid. Steve's comments reflected rather unkindly upon the microbial contamination of an extract in which he had invested much time and effort…. »

*Discovery of Bacterial Ice Nucleation. G. Vali*


Фото Игоря Микулёнка.

# Сказка об ОХОТЕ на НЕВИДИМЫХ ГРАБИТЕЛЕЙ, орудующих ЛЕДЯНЫМИ КИНЖАЛАМИ

Ник ГОРЬКАВЫЙ.

**Умная девочка Никки — героиня научно-фантастических романов Ника Горькавого «Астровитянка», «Теория катастрофы» и «Возвращение астровитянки» — выросла на астероиде под присмотром искусственного сверхинтеллекта, а потом попала в человеческое общество, как жук в муравейник. Прочитав трилогию, вы узнаете о том, какие приключения выпали на долю этой бесстрашной девочки и кто помог ей выжить в самые трудные моменты. Один из критиков даже назвал Никки «героиней нашего времени», под впечатлением от её образа он написал: «Если хотите преуспеть в этой жизни, жаждете добиться чего-нибудь стоящего, учитесь, мечтайте, дерзайте».**

**Автор этих книг Николай Николаевич Горькавый (Ник Горькавый — это его псевдоним) — астрофизик, доктор физико-математических наук, писатель, соавтор двух детских энциклопедий: «Энциклопедия для детей. Том 8. Астрономия» и «Большая детская энциклопедия. Вселенная». Он убеждён, что мир принадлежит умным и образованным. Сейчас Ник Горькавый заканчивает работу над сборником «Звёздный витамин». И мы предлагаем вам познакомиться с некоторыми «научными сказками» из этой книги.**

— Сегодня время интересной сказки о воде, обыкновенной воде, — так начала свою традиционную вечернюю историю принцесса Дзинтара, которая была не только принцессой, но и учёным-биологом.

— Что в воде может быть интересного? — удивлённо спросила младшая Галатея. — Вода — она и есть вода, мокрая и пить можно.

— Вода — одно из самых загадочных веществ на Земле, — возразила Дзинтара. — Например, при какой температуре вода замерзает?

— При нуле градусов! — выкрикнул старший Андрей. — Так учитель говорил.

— Учитель прав и неправ одновременно, — кивнула принцесса. — На самом деле, если поставить воду в холодильник, то она может остаться жидкой и при нескольких градусах ниже нуля. Такая переохлаждённая

● **РАССКАЗЫ О НАУКЕ**

Ice nucleation active bacterial species

---

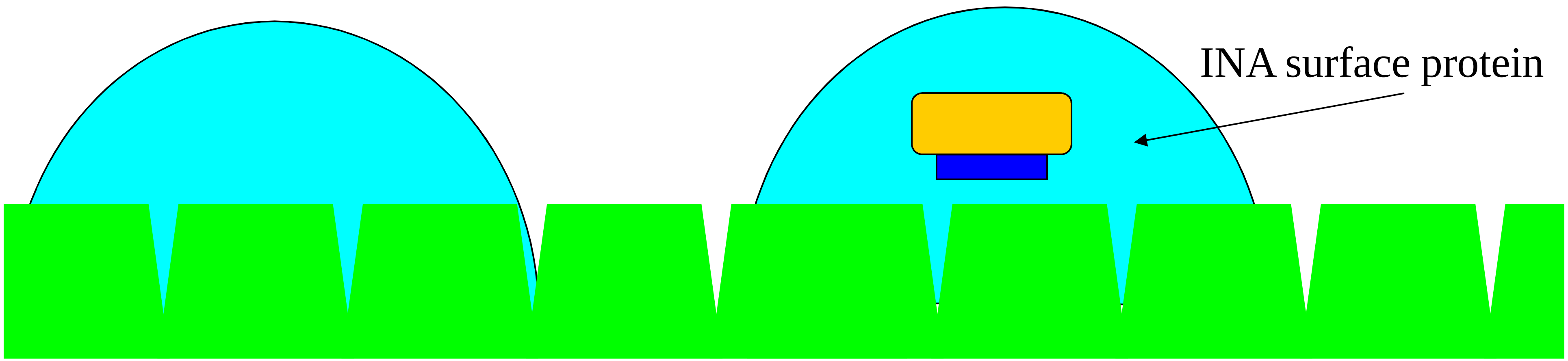
*Pseudomonas syringae* (ca. 1/2 of all pathovars)

*Erwinia herbicola* (only about 10% of strains?)

*Pseudomonas fluorescens* (only about 1% of all strains?)

*Pseudomonas viridiflava* (common?)

*Xanthomonas campestris* pv. *translucens*



## Ice nucleation active bacterial species

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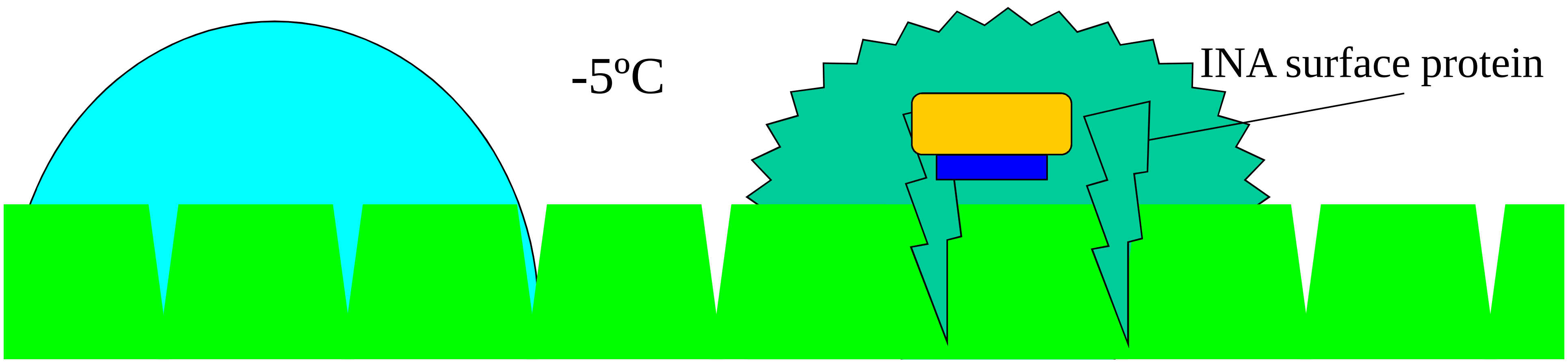
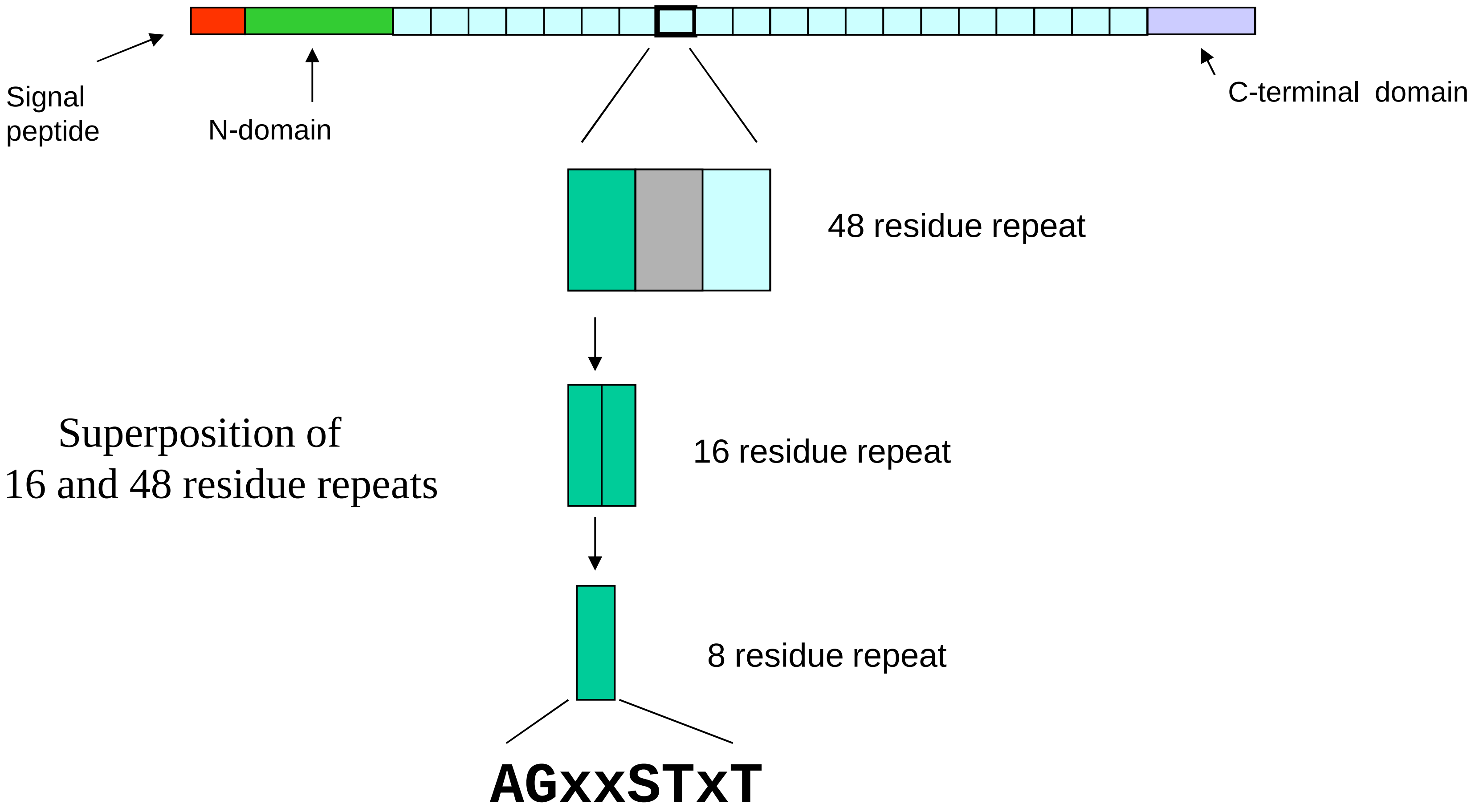


# Ice nucleation protein ~1200 residues




Superposition of
8, 16 and 48 residue repeats

# Secondary structure prediction
# Beta-structural protein

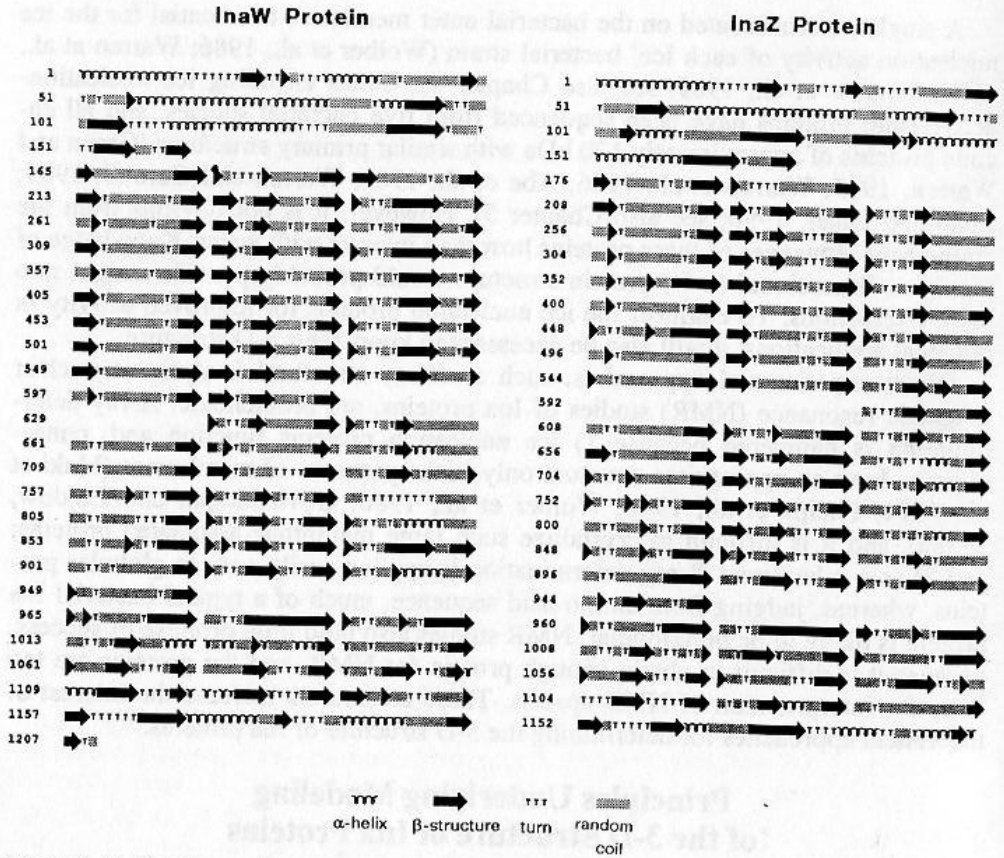
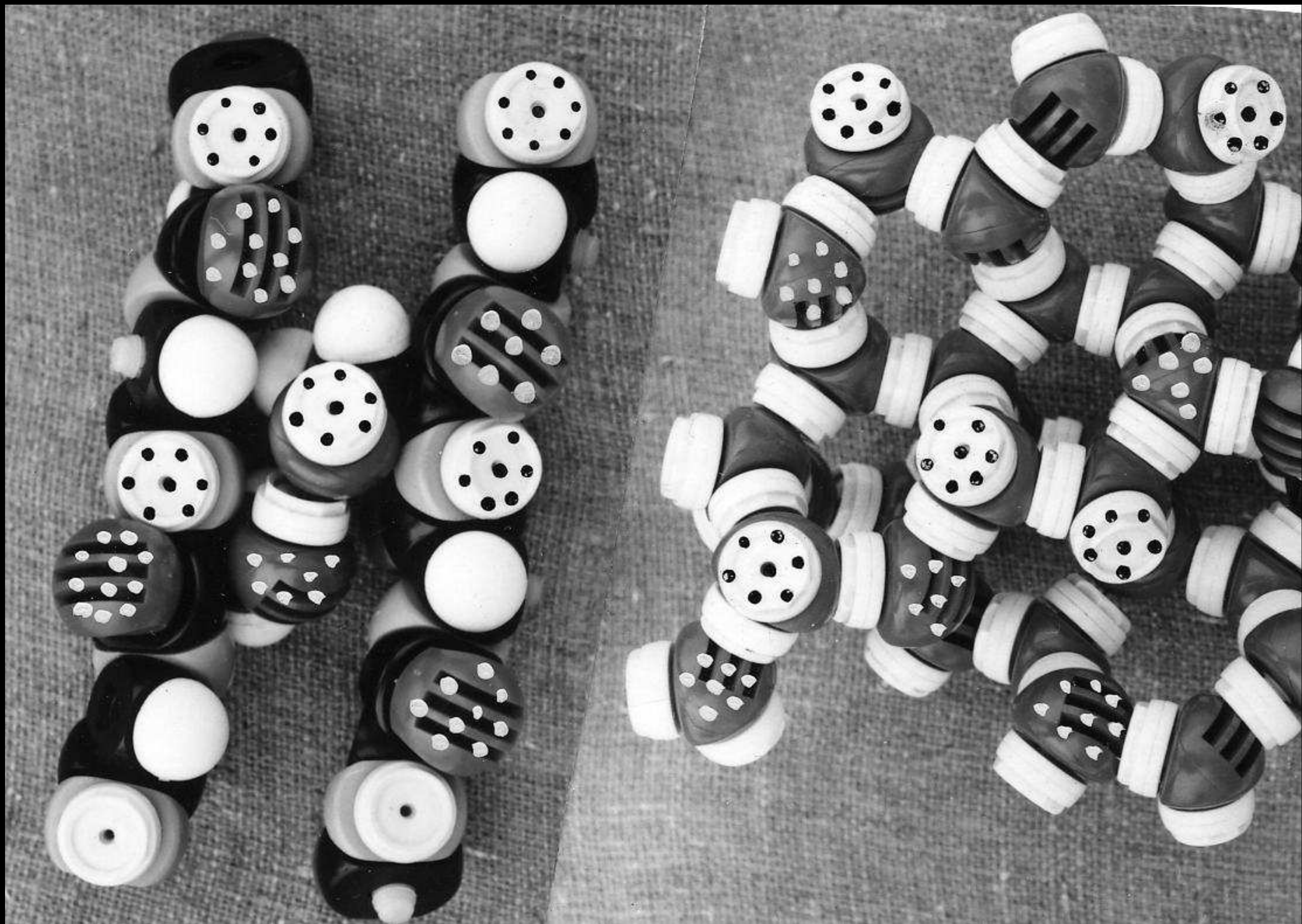



**Figure 1.** Predicted secondary structures of the InaW and InaZ proteins. The layout of the predictions is isometric with that of the amino acid sequences (see Chapter 5). Reprinted with permission from Warren et al. (1986).

# THR-, SER-RICH REGIONS OF BACTERIAL ICE-NUCLEATION PROTEIN



Institute of Protein Research
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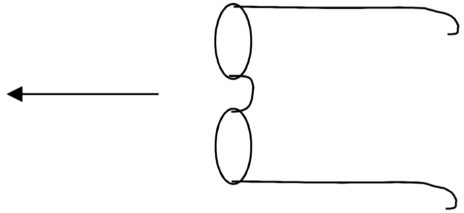
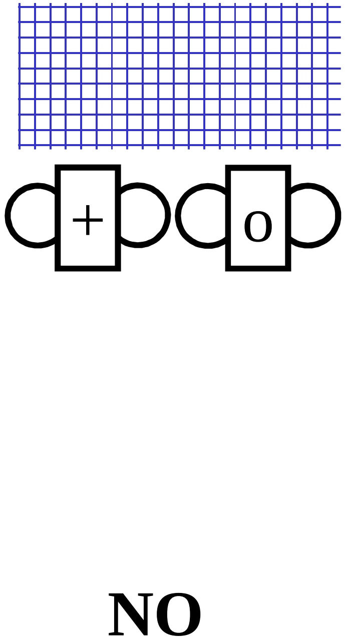
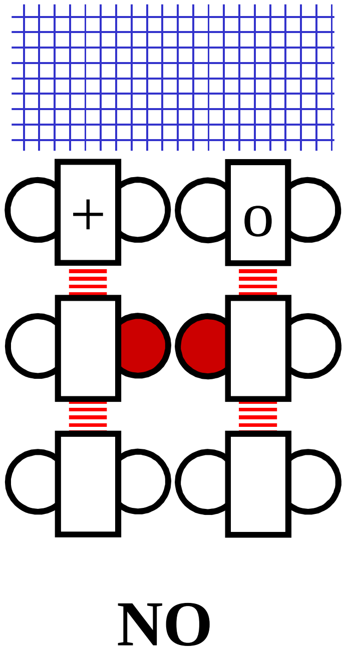
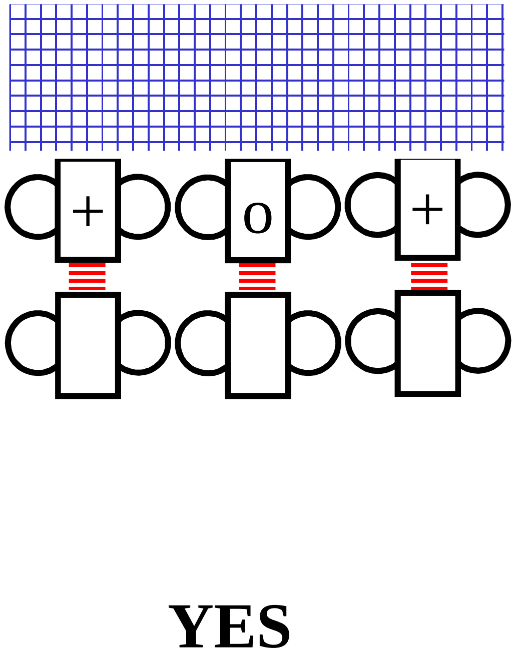
Berkeley



AGxxSTxT – less than 30% of apolar residues



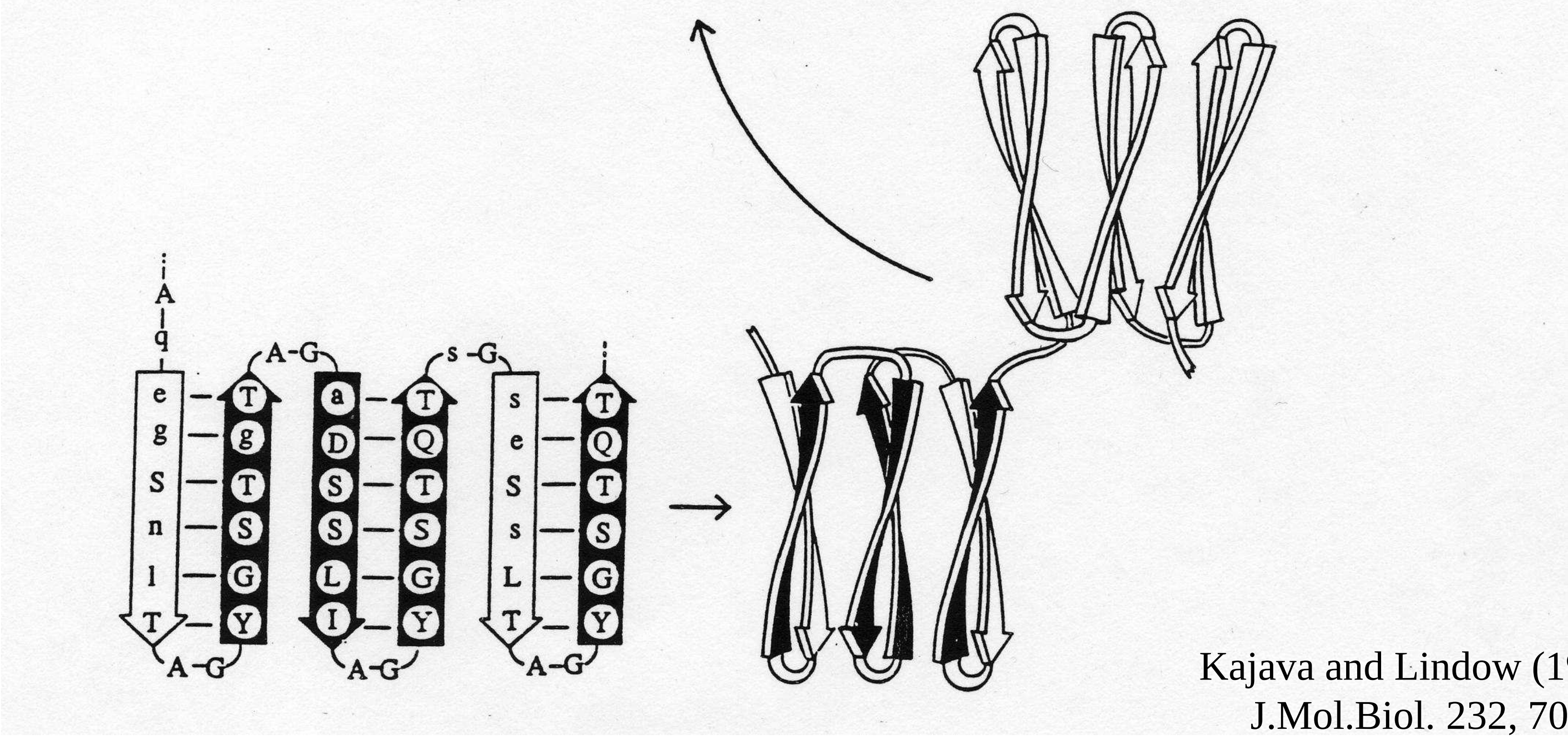
1. Has 8-, 16, and 48-residues periodicities

2. Gly are in turns, Thr and Ser in the middle of beta-strands and form ice-like surfaces

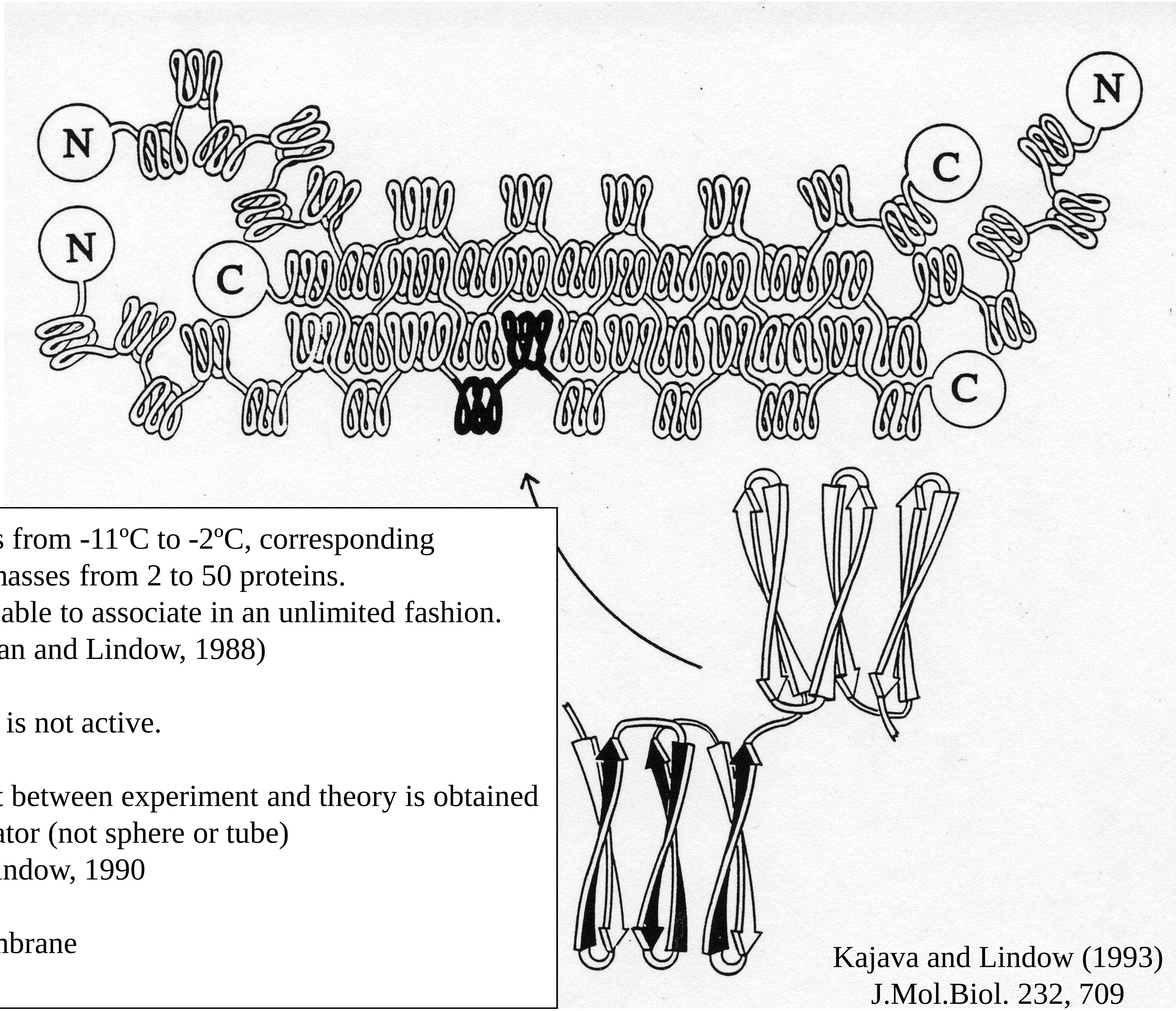
3. One repeat does not have Gly and this repeat does not produce a turn.

4. The lenght of the beta-strands – average of known proteins

5. 48-residue block: four highly conserved octapeptides and two low conserved octapeptides



Kajava and Lindow (1993)
J.Mol.Biol. 232, 709




1. INA occurs from -11ºC to -2ºC, corresponding
to nucleant masses from 2 to 50 proteins.
INP must be able to associate in an unlimited fashion.
(Govindarajan and Lindow, 1988)

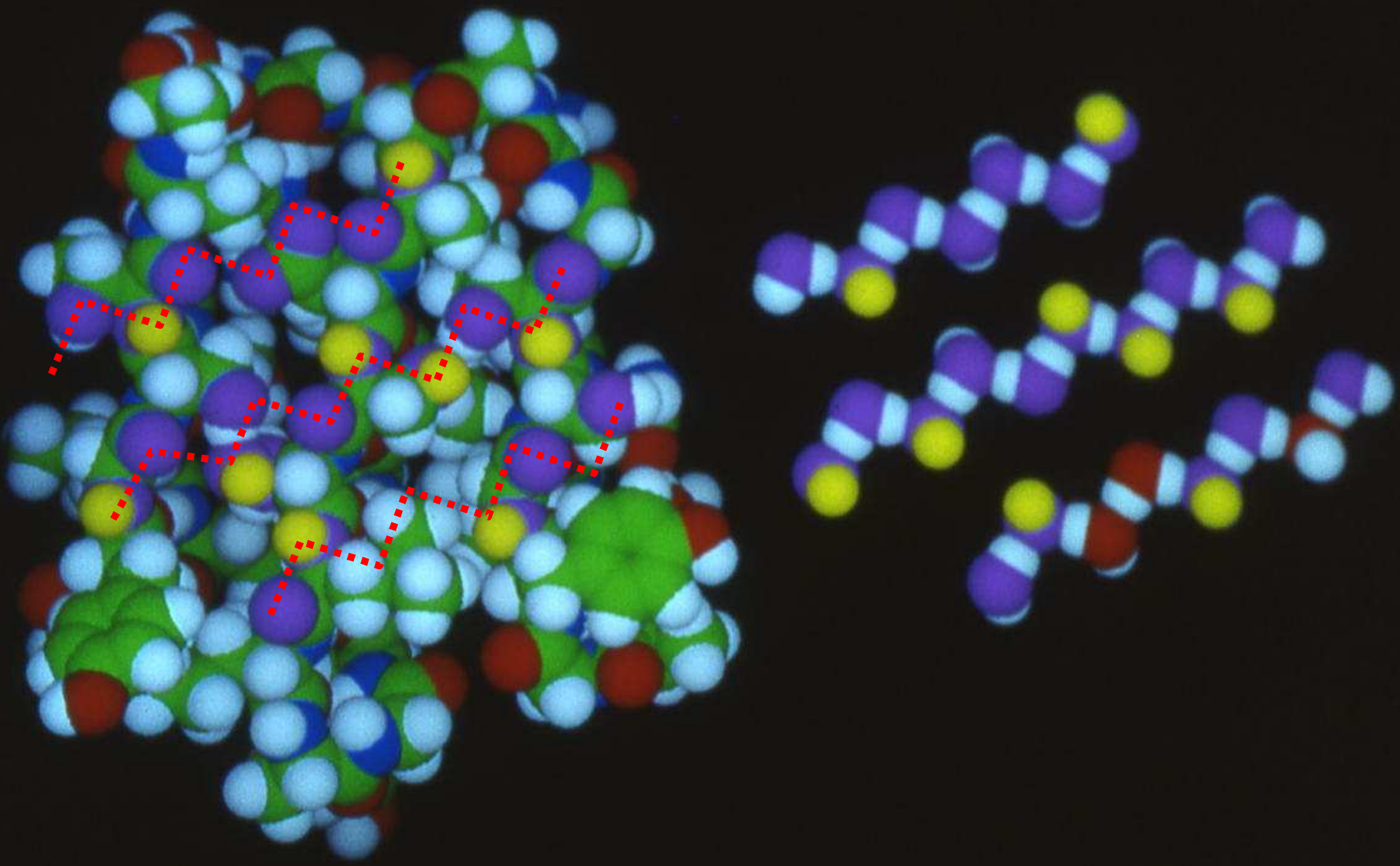
2. Single INP is not active.

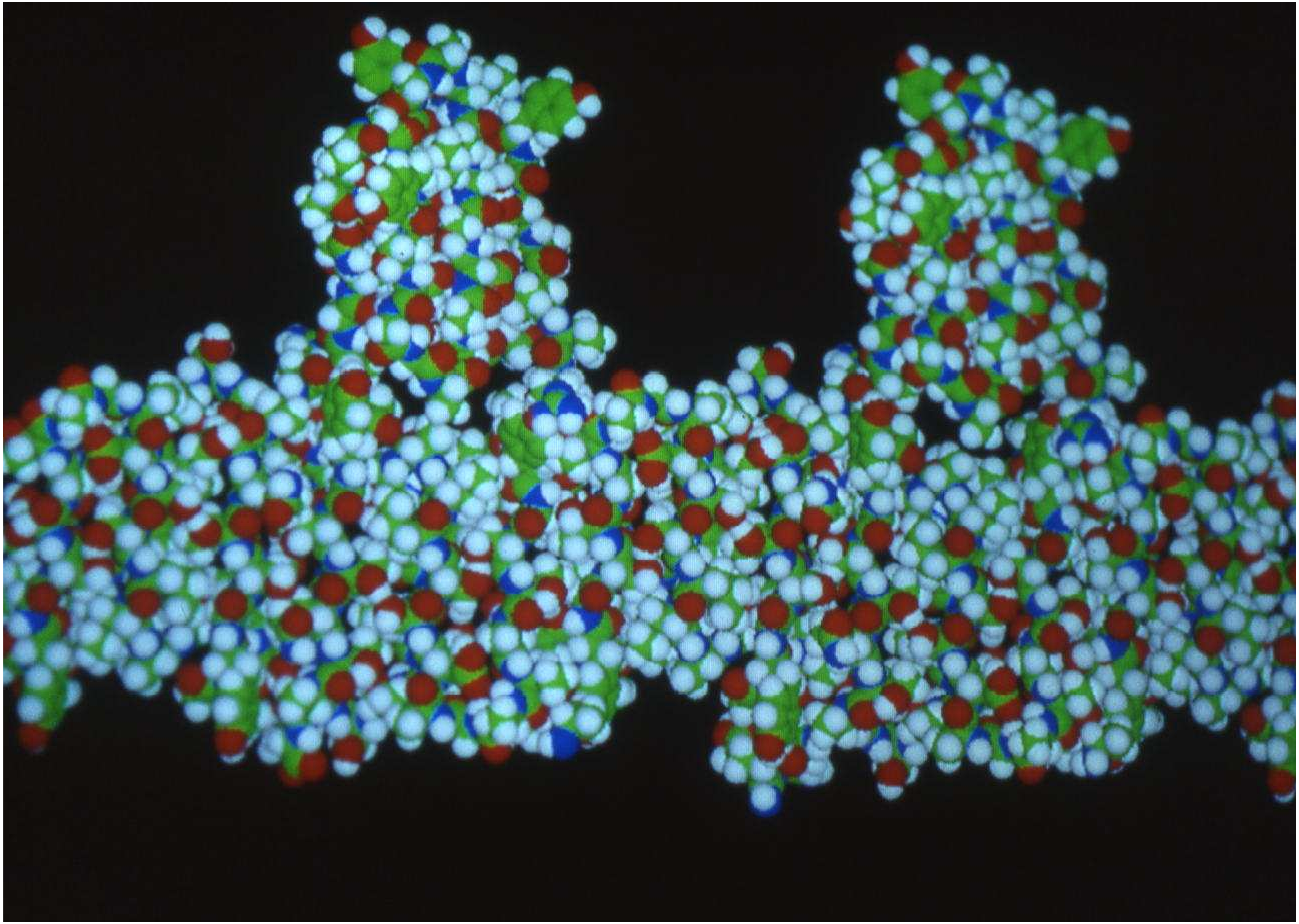
3. The best fit between experiment and theory is obtained for flat nucleator (not sphere or tube)
Burke and Lindow, 1990

4. Needs membrane

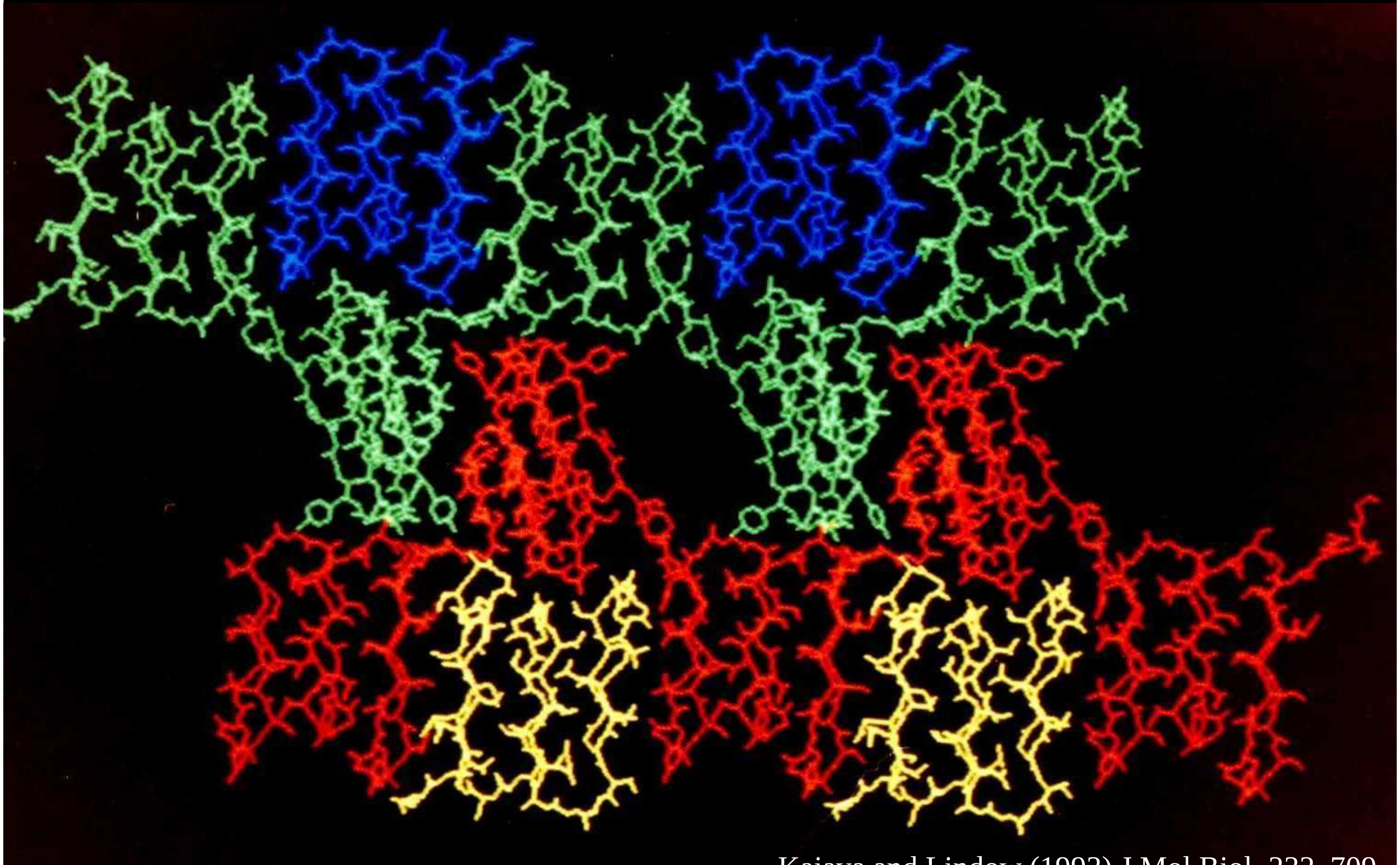
Kajava and Lindow (1993)
J.Mol.Biol. 232, 709

# 48-residue block



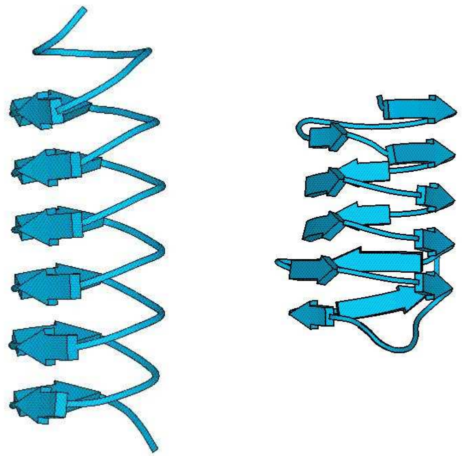


# A MODEL OF ICE-NUCLEATION PROTEIN



Kajava and Lindow (1993) J.Mol.Biol. 232, 709

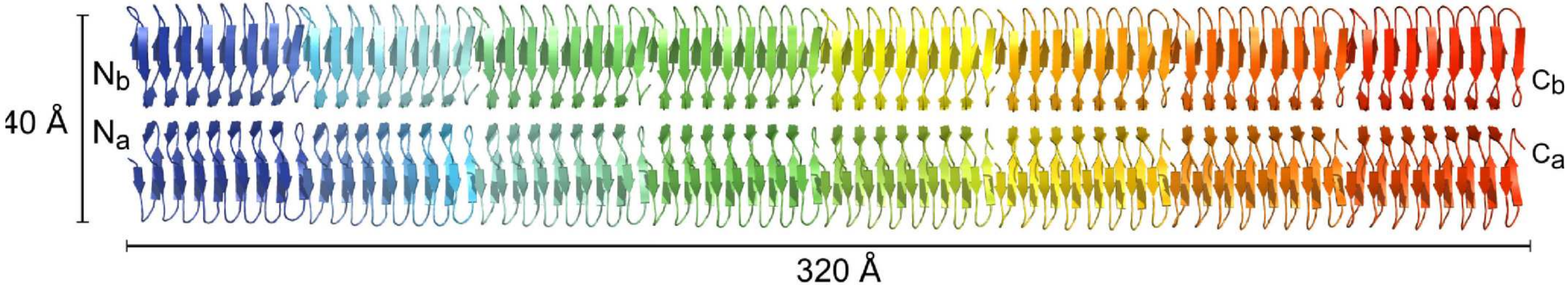
Antifreeze proteins



Liou et al. 2000, Nature, 406, 322
Leinala et al. 2002, J. Biol.Chem

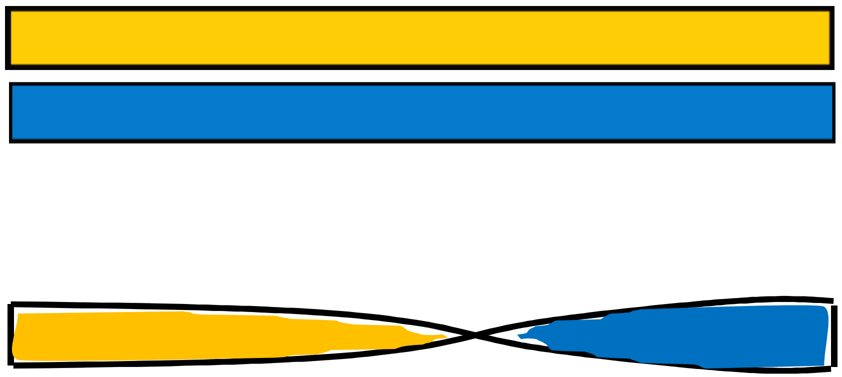
Beta-helical InP model

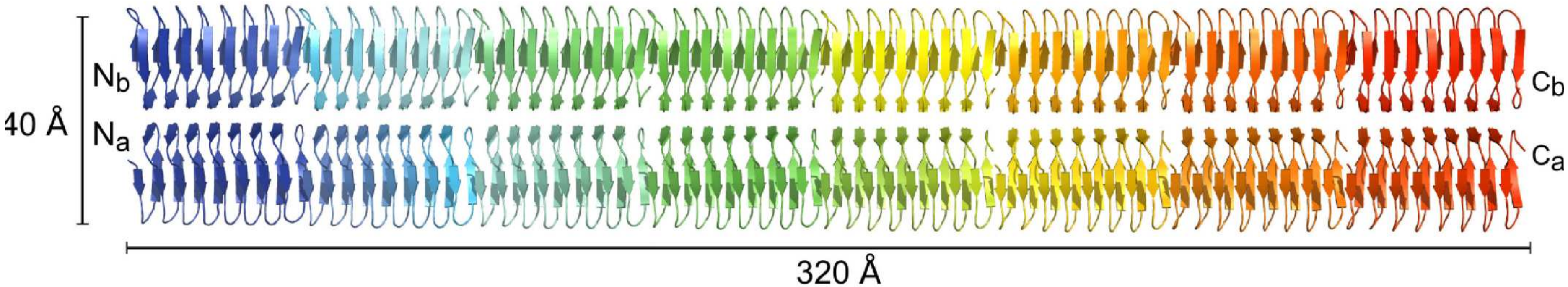

Graether and Jia (2001)Biophysical J 80, 1169




Garnham et al., BMC Struct Biol. 2011 Sep 27;11:36.

# INP is functional when bound to the membrane






Garnham et al., BMC Struct Biol. 2011 Sep 27;11:36.

# Some applications of INP

Agriculture

Control of temperature-sensitive materials (Vaccines)

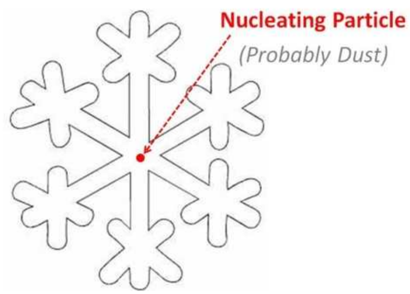
Climate, ecology

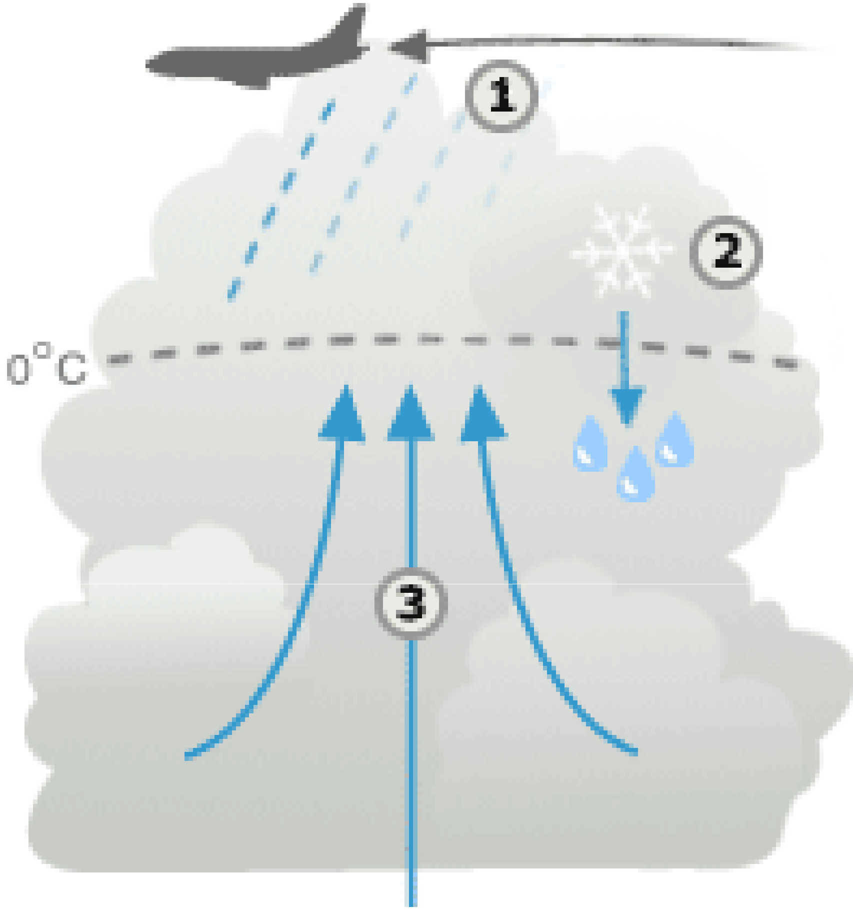
Artificial snow

Food industry

Saving of energy

# Applications of INP




**Climate, ecology**

Artificial snow

Food industry

Control of temperature-sensitive
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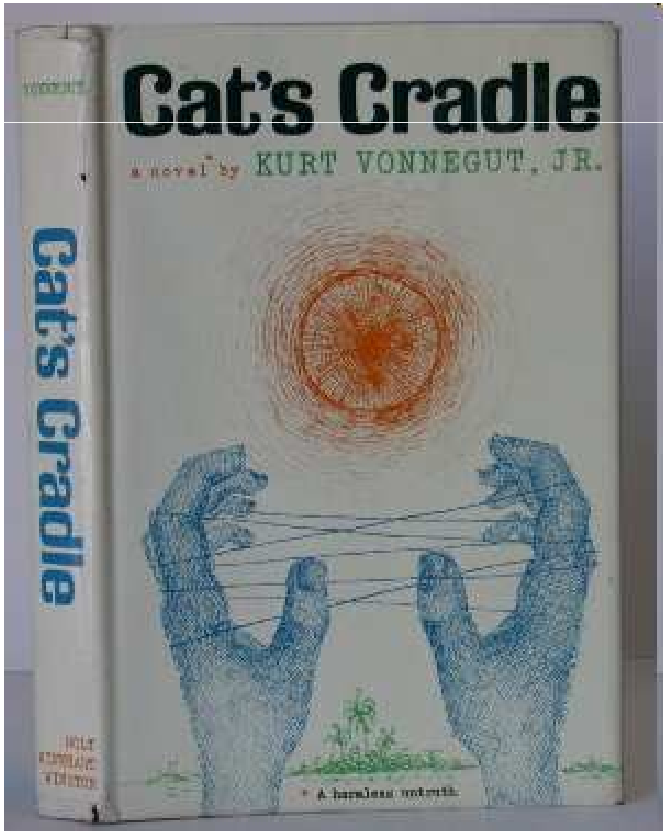
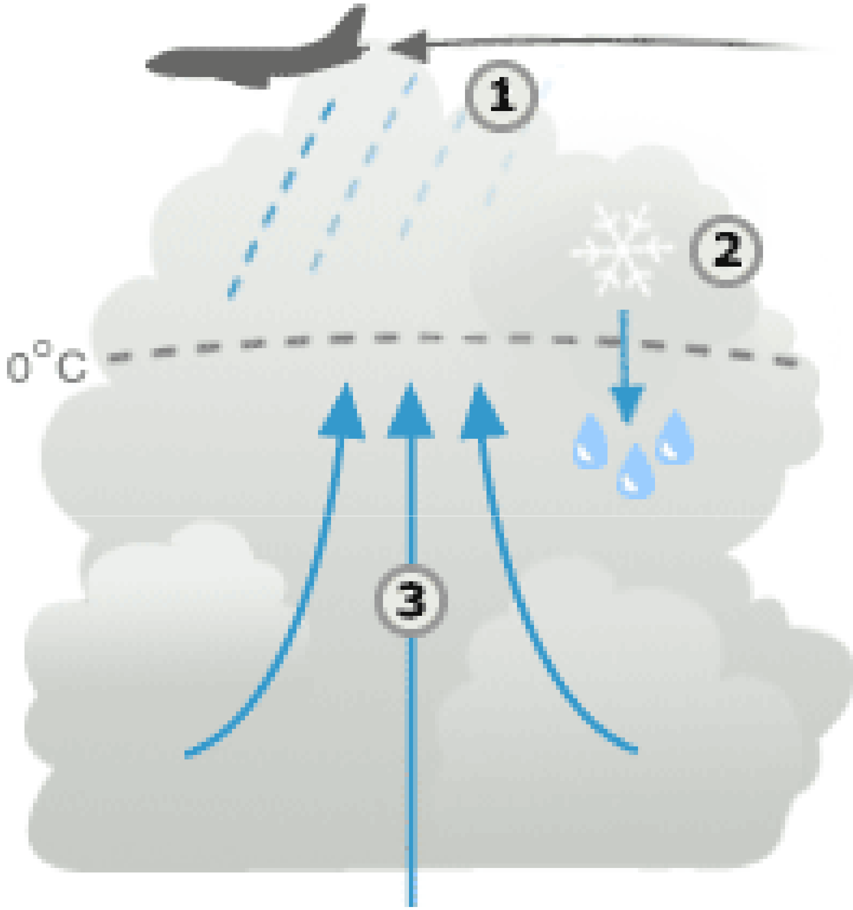
Saving of energy

# Applications of INP

**Climate, ecology**



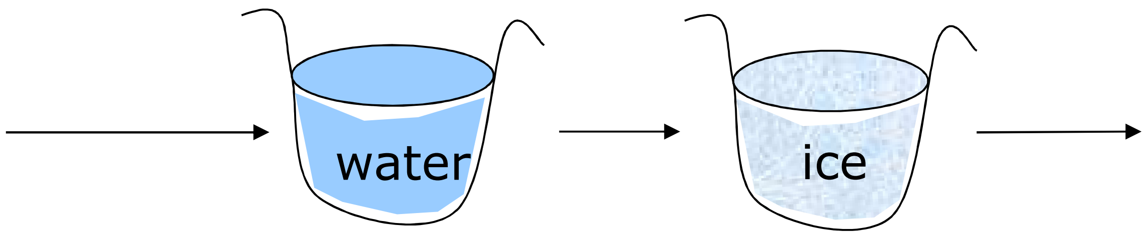

Ubiquity of biological ice nucleators in snowfall.
Christner et al. *Science*. 2008 319(5867):1214.

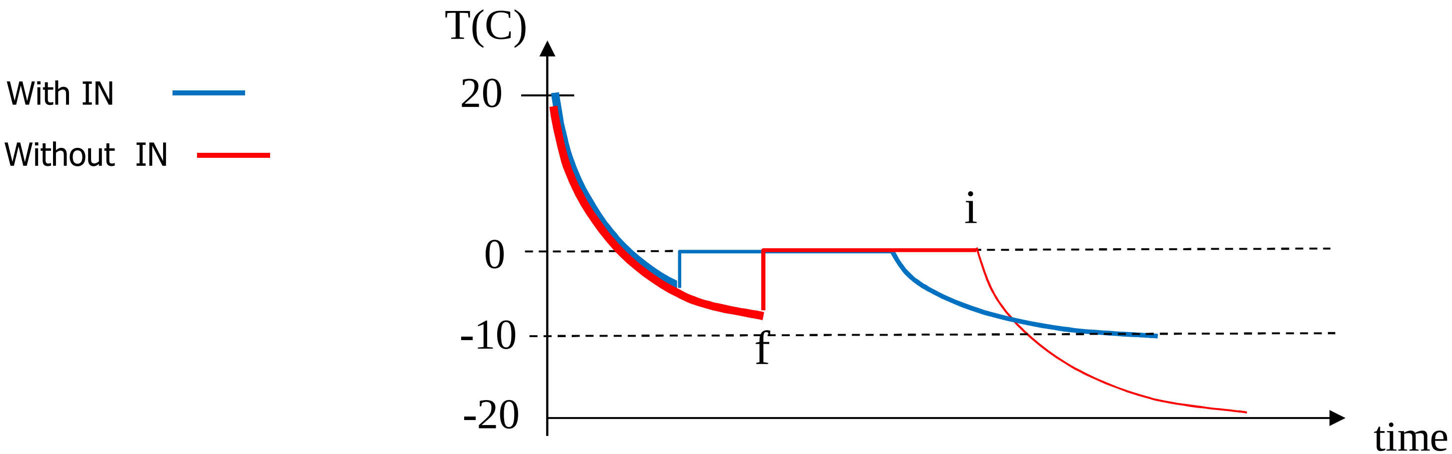
# Applications of INP

**Artificial snow**


**Saving of energy** when using ice-nucleators in the process of freezing of water

# Industrial process with freezing step



## COLLABORATORS

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