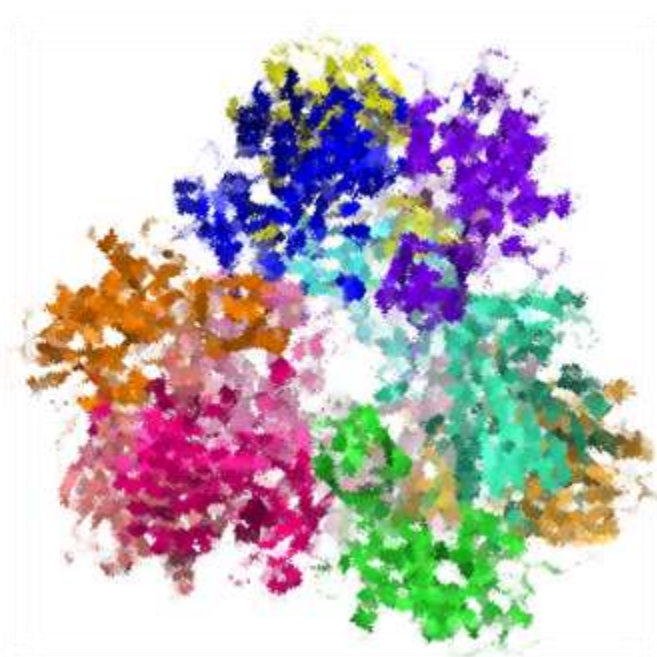
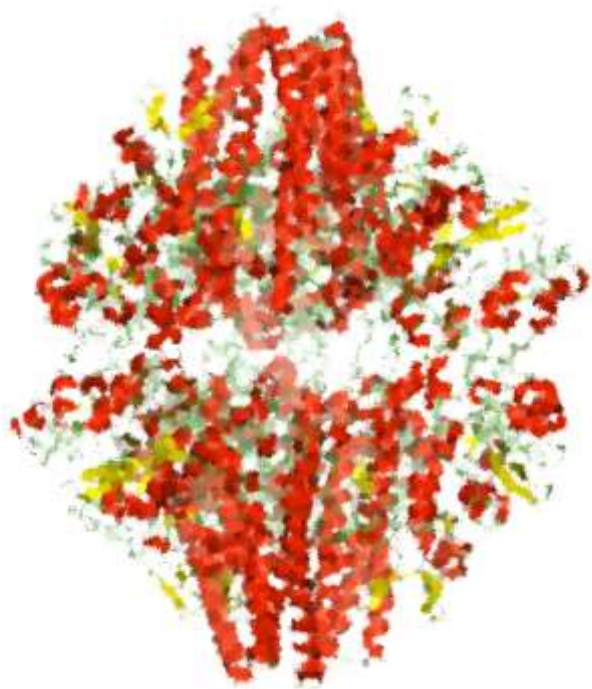


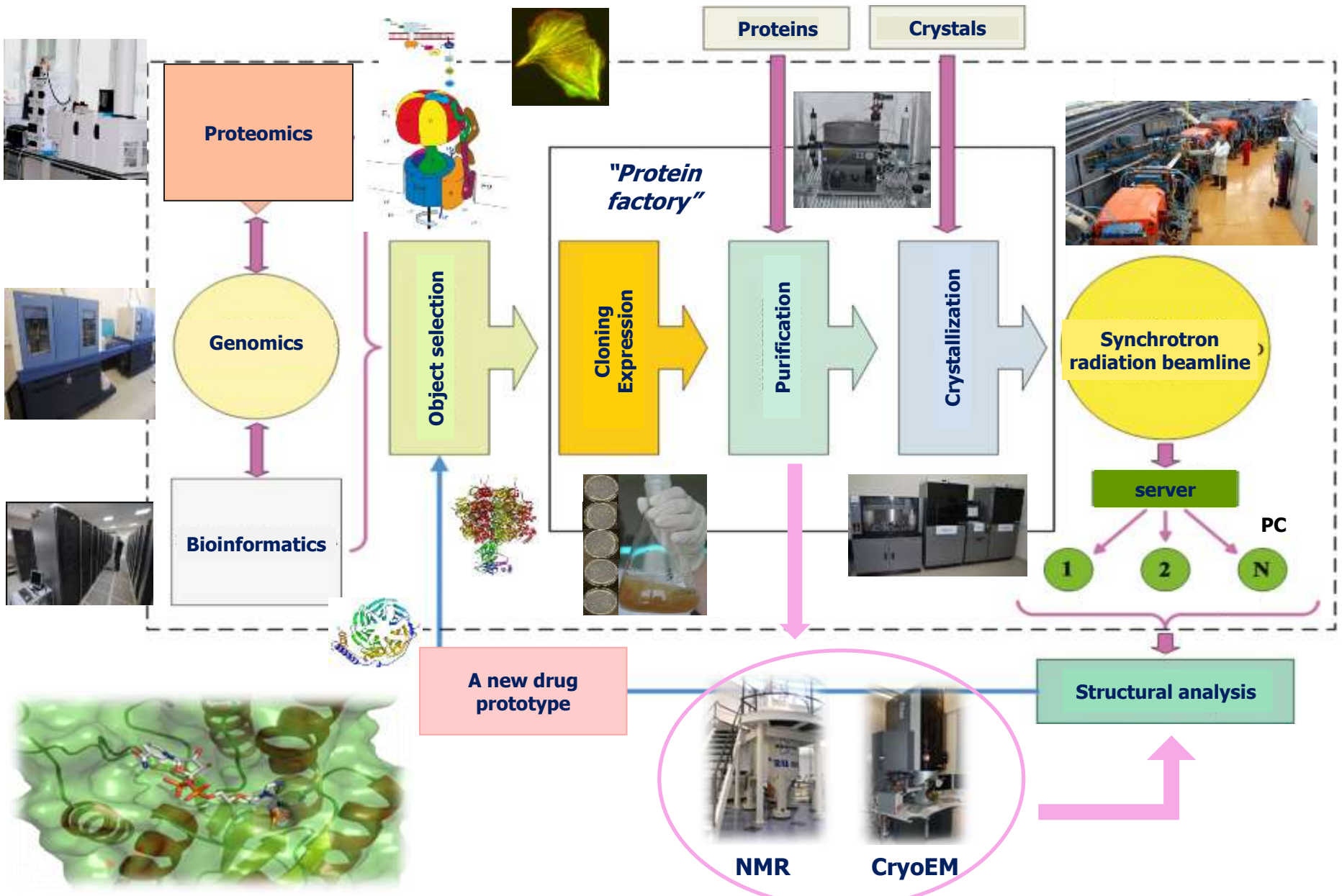
National Research Center "Kurchatov Institute" NBICS-Center



Protein Factory



Structure of protein factory

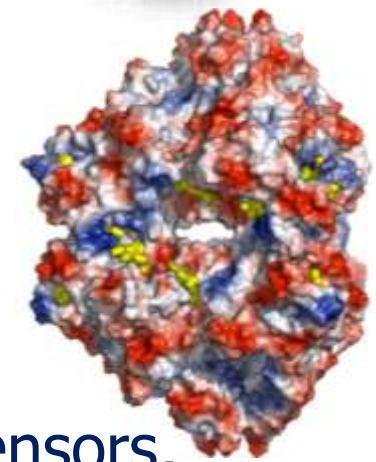


“Protein Factory” areas of interest

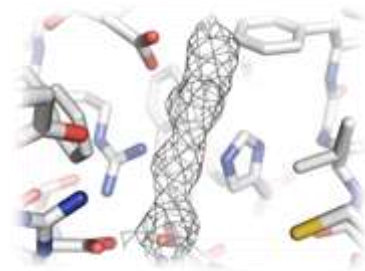
- Enzymes for biotechnological applications (e.g. enzymes from extremophilic organisms)



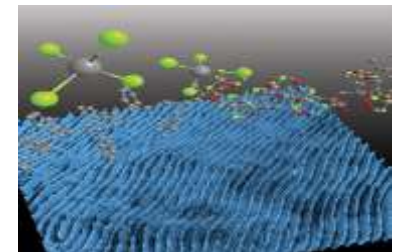
- Large protein complexes and nano-machines



- Drug design

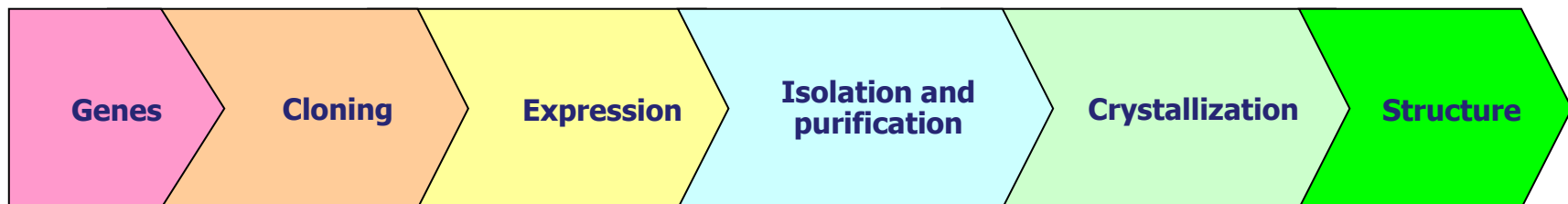


- Nano-biocomposite materials and devices (sensors, biofuel cells)



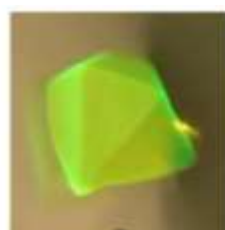
Competences and facilities

- **Expression systems**
 - *Bacterial*
 - *Yeast*
 - *Baculovirus*
- **Scaling up and isolation**
 - *Fermenters with working volume up to 6 l (up- and downstream)*
 - *Micro- and ultrafiltration*
 - *HPLC, FPLC*
- **Analytical systems**
 - *MALDI TOF/TOF*
 - *LC/MS, GC/MS*
 - *DSC, ITC, Biocore, DLS, CD*
 - *TEM, SEM, (Electron microscopy center of Kurchatov Institute) AFM, confocal*
- **Crystallization**
 - *Robotic crystallization system*
 - *Microgravity crystallization in space*
- **Data acquisition**
 - *Kurchatov synchrotron, station «Belok»*
 - *Collaborations*
 - *DESY, Germany*
 - *SPRING8, Japan*
 - *ESRF, France*
 - *ARGONNE, US*
 - *NMR center of Kurchatov Institute*
 - *CryoEM facilities of Kurchatov Institute*
 - *XFEL at EMBL (Hamburg)*



Automated crystallization system

- Minimum dispense volume – 100 nl of protein per well
- Two incubators store up to 1400 plates simultaneously
- Storage of crystallization plates at specified temperature (standard: +4 or +15 C)
- About 1 minute to dispense one 96-well plate
- Eliminated “human factor”
- Results could be monitored via remote access
- Initial step prior to manual optimization



Remote access to the crystallization results

Rigaku CrystalTrak Web v2.2.4 Results - Optimization User: Kostya Update Password Log Off

View: Kostya

Plate: Drop Slice: Best Focus Type: 00 - Normal Colists: Last Subwells: 3 Thumb: XOL

APH VIII: RG000060 (8), R11.3
4* / Scored Crystals - harvestable

Precipitate

Crystal

Other

Plate Notes: RG000060

Drop Conditions [33.33% APH VIII 21.07.11, 0.3µl]

Type	Conc.	Units	Name	pH	Drop	Source
Sample	12.5	mg/ml	APH VIII		Protein	APH VIII 21.07.11
	0.5	mM	magnesium chloride	7.8	Salt	APH VIII 21.07.11
	40	mM	sodium chloride		Salt	APH VIII 21.07.11
	20	mM	Tris	7.8	Buffer	APH VIII 21.07.11
	3	mM	glycerol		Organic	APH VIII 21.07.11

Score History

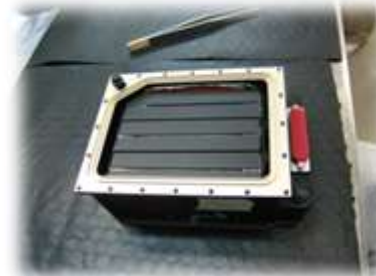
Score	Ins	By	Date
Crystals - harvestable	1	Kostya	8/4/11 6:22p

Update

Protein crystal growth in space (under microgravity conditions)



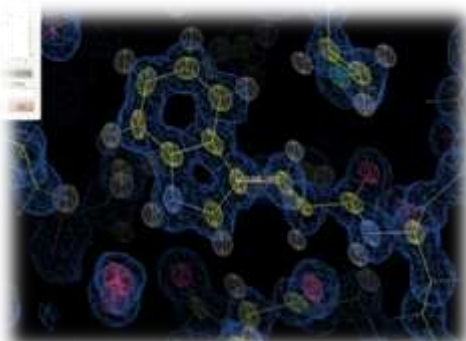
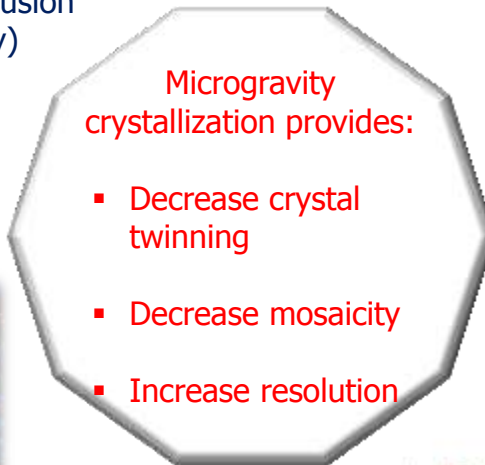
Transferring from vapor diffusion to counter diffusion method (in capillary)



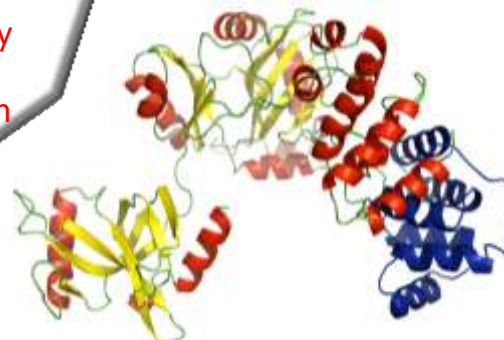
Special module for microgravity crystallization



Crystal growth for 1-2 months on ISS



Uridine phosphorilase, 0.95A
Space-grown crystals, 0.95



DNA-ligase, 2.80 A
New structure details revealed



X-Ray experiment after landing

In-house X-ray beamline

Protein crystallography station at synchrotron source of
“Kurchatov Institute” NBICS Center



- Channel 3.4. Length 26 m, Wiggler 3T, 69 poles
- Size of a beam focused on the crystal
 $H \times V = 0,3 \times 0,3$ mm
- Flux of radiation: 10^{13} photon/c - composite monochromator
- Energy resolution: $\Delta\lambda/\lambda < 2 \cdot 10^{-4}$ for Si(111) composite monochromator

Major collaborations:

- Institute of Crystallography RAS
- Moscow State University
- Institute of Molecular Biology RAS
- Institute of Gene Biology RAS
- Institute of Bioorganic Chemistry RAS
- Institute of General Genetics RAS
- Moscow Institute of Physics and Technology
- Petersburg Nuclear Physics Institute
- Institute of Protein Research RAS

- **European Molecular Biology Laboratory (Hamburg, Germany)**
- **Spring8 (Harima, Japan)**
- **ESRF (Grenoble, France)**
- **Argonne (Chicago, USA)**
- **University of Virginia (USA)**



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