Protein Production Pipeline

- Target Filtering Based on Amino Acid Sequence, Homology and Availability
- Protein Cloning, Expression and Purification
- 1D $^1$H NMR Spectra Acquisition and Fold Assessment
- Structure Determination

Core

- Bioinformatics
- Crystallomics, NMR
- NMR
- NMR (Xstallo)
NMR Screening Setup

1 mm diameter capillary tube vs 5 mm diameter tube

600 μL  5 μL

Gilson Liquid Sample Handler

Bruker Automated Sample Changer
NMR Spectra with Micrograms of Protein

PC07372C
From Silicibacter sp
MW: 12.3 kDa
NMR Grade: A

PC04405A
From: E. carotovora atroseptica
MW: 24.9 kDa
NMR Grade: D

- Only ~30 μg of protein needed.
- Spectra were acquired fully automatically.
- 20’ measurement time/spectrum  ➔ ~70 samples/day
SARS CoV: nsp3c ("SARS-unique")

Chatterjee, A., Johnson, M.A., work in progress

Nsp3c(451–651)

Nsp3c(513–651)
Micro-coil $^1$H NMR (7 µl) of GPCRs:

β2 Adrenergic Receptor/Dodecyl Maltoside

(B. Kobilka; R. Stevens; R. Horst)
1D $^1$H NMR Diffusion Experiments

- $\text{In}(S/S_0)$ vs. $G^2d^2g^2(\Delta-\delta/3) \times 10^{-5}$ [cm$^2$/s]

Legend:
- Sample A, detergent signal
- Sample A, protein signal
- Sample B, detergent signal
- Sample B, protein signal
NMR Studies of the Solvation of Membrane Proteins in Detergent Micelles
OmpX from *E. coli* in DHPC Micelles

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NMR structures of proteins

OmpX/DHPC 60 kDa
$[^2\text{H},^{15}\text{N}]$-OmpX/DHPC / pH 6.8 / 303K
TROSY

1997 –

Transverse Relaxation-optimized Spectroscopy
Solution NMR structures of membrane proteins

OmpX  OmpA  PagP
Production of membrane proteins in *E. coli* for NMR studies

- Unfolded protein in inclusion bodies
- Native protein in cell membrane
- *In vitro* solubilization, refolding, and reconstitution in detergent micelles
- Extraction and transfer into detergent micelles
Mix 1 volume of unfolded OmpX (0.6 mM in 6 M urea) with 6 volumes of detergent-containing “refolding buffer”. Stir overnight at 4 °C.

Precipitated protein can be recovered with 6 M urea

Recover supernatant containing detergent-refolded Omp X.

Exchange into detergent-containing “NMR buffer” by way of concentration/dilution.

Concentrate to 50 µL.

Micro-probe 1D 1H NMR screening
OmpX/DHPC

intramolecular and intermolecular NOEs
$\text{NOE} \propto r_{ij}^{-6} f(\tau_c)$
Selective methyl group protonation: $V,L,I(\delta^1)$
Lipid–protein interactions in OmpX/DHPC
Lipid–protein interactions in OmpX/DHPC

OmpX/DHPC micelles

DHPC bilayer

E. coli OM

16 Å

28 Å
OmpX/DHPC, Gd(DOTA)

$[^{15}\text{N},^{1}\text{H}]-\text{TROSY}$ of OmpX

$\omega_1(^{15}\text{N})$ [ppm]

$\omega_2(^{1}\text{H})$ [ppm]

$1\text{D} ^{1}\text{H} \text{NMR}$ of DHPC

$\omega_1(^{1}\text{H})$ [ppm]
OmpX/DHPC, Gd(DOTA)
Relaxation enhancement $\varepsilon$
Intermolecular NOEs  OmpX–DHPG