Structural studies of the methyltransferase NlaX

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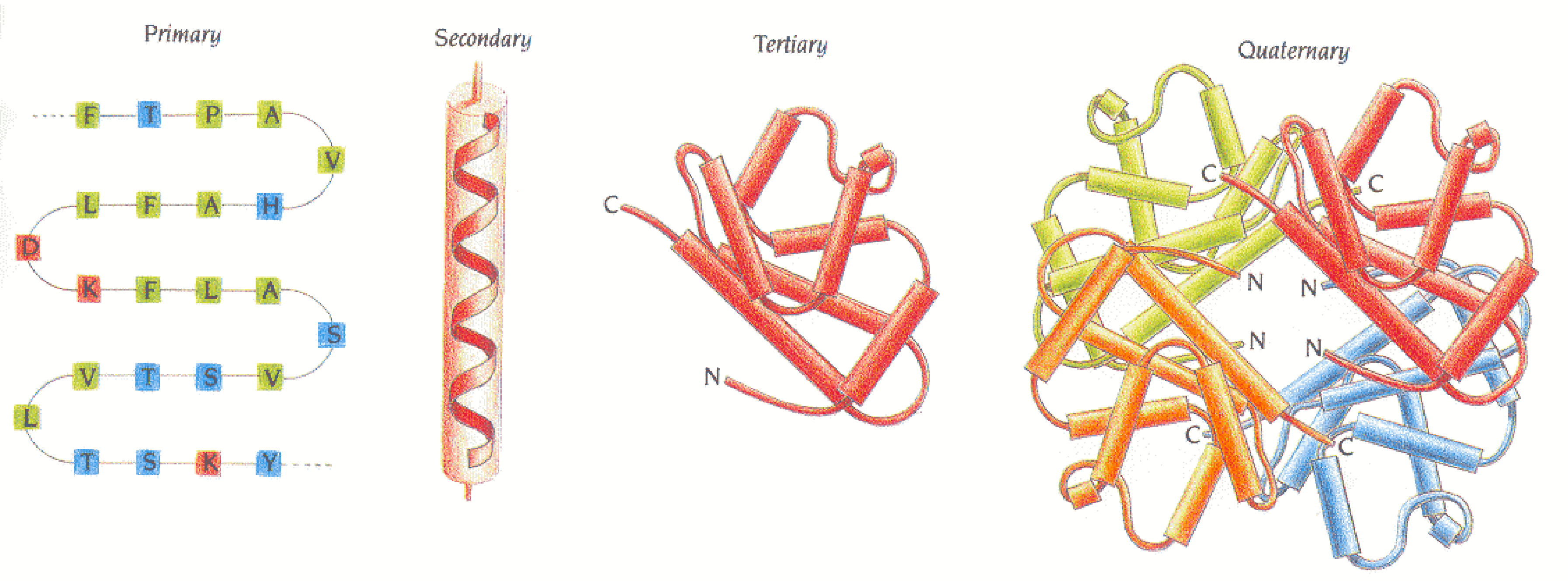
Protein structures



NlaX

Neisseria lactamica: 34.8 kDa,
313 amino acid residues
Expression in *E. coli:* 36.5 kDa,
6xHis tag on the N-end

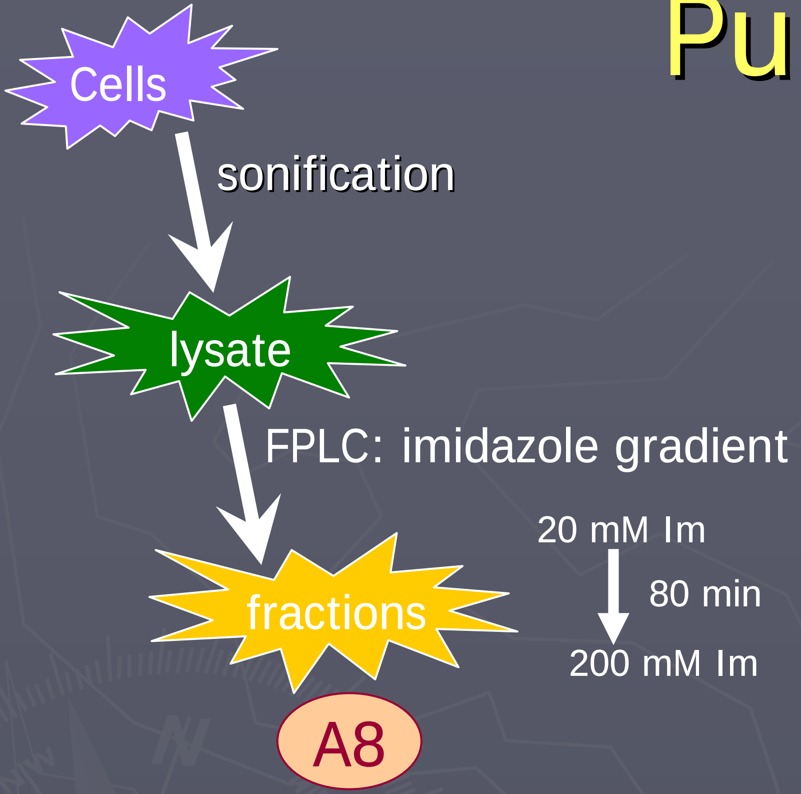
C5-cytosine-specific methyltransferase:

methylates the C5-atom of cytosine in the recognition sequence

5′ – CCNGG – 3′
3′ – GGNCC – 5′
(N = A, G, T, C)

S-adenosyl-L-methionine + DNA
↓
S-adenosyl-L-homocysteine +
DNA containing 5-methylcytosine

Purification



Cells

sonification

lysate

FPLC: imidazole gradient

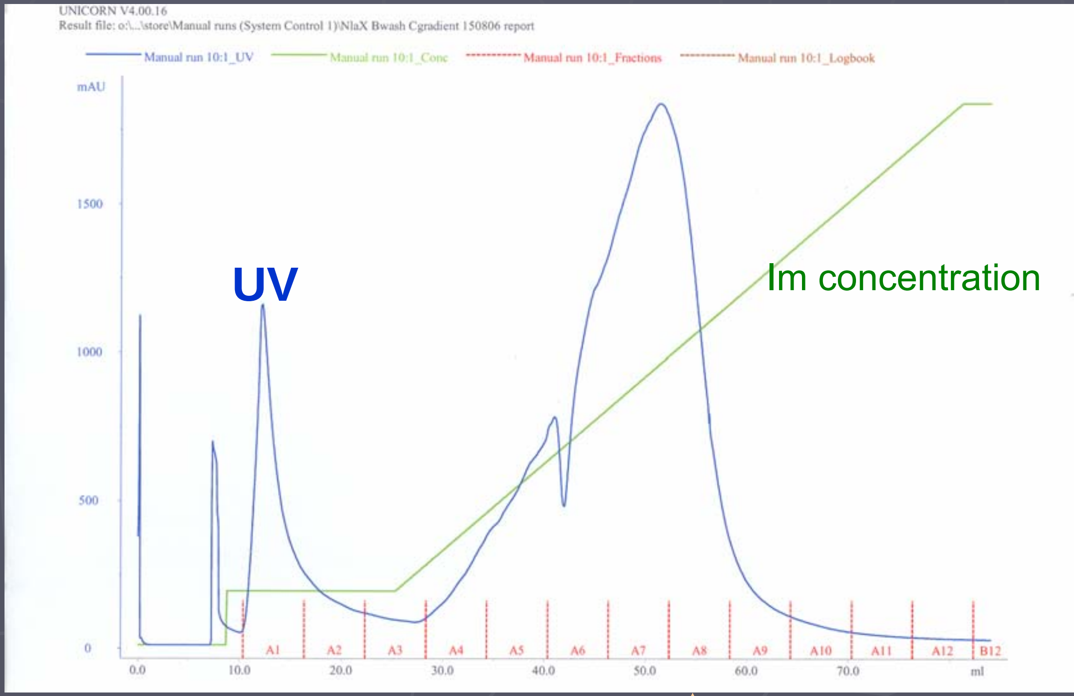
fractions

20 mM Im

80 min

200 mM Im

A8



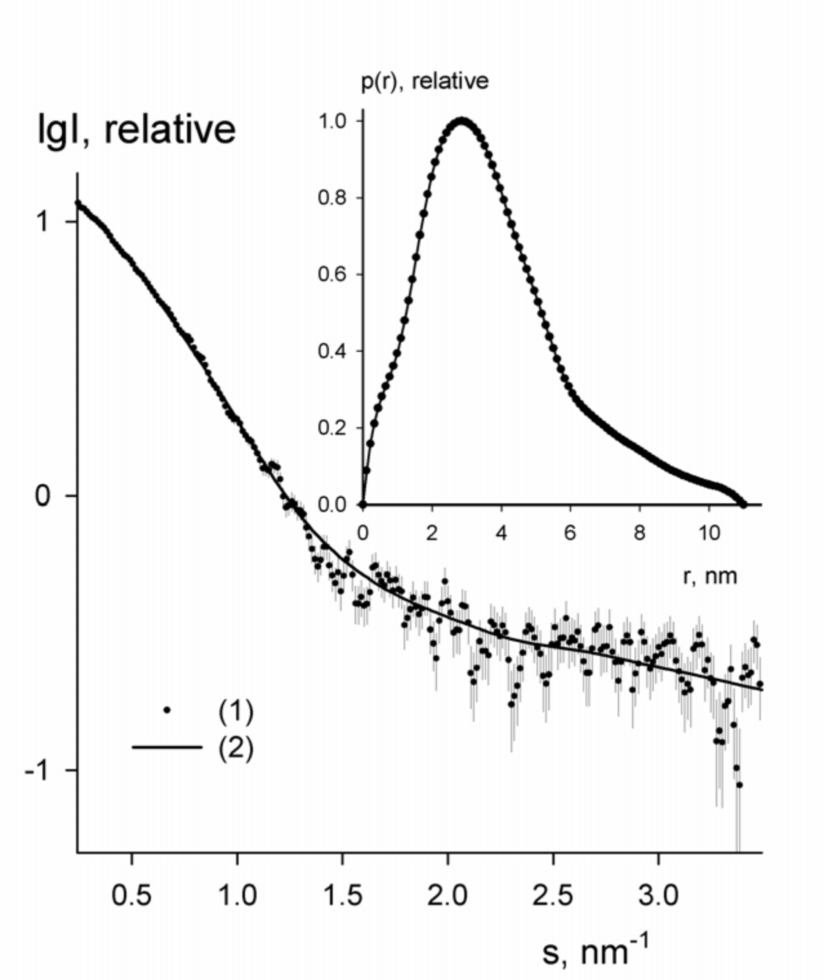
UNICORN V4.00.16
Result file: o:\...\store\Manual runs (System Control 1)\NlaX Bwash Cgradient 150806 report

A8

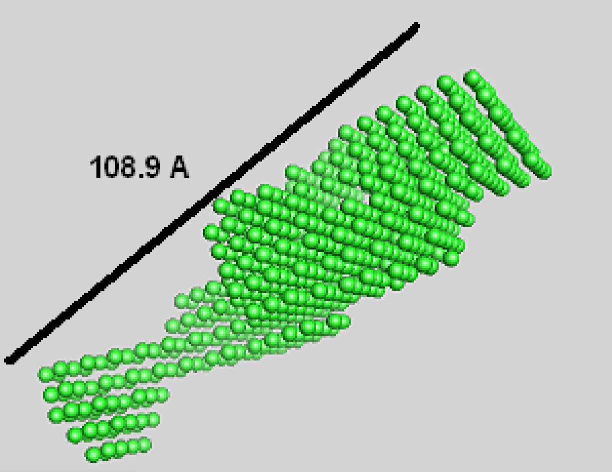
SAXS

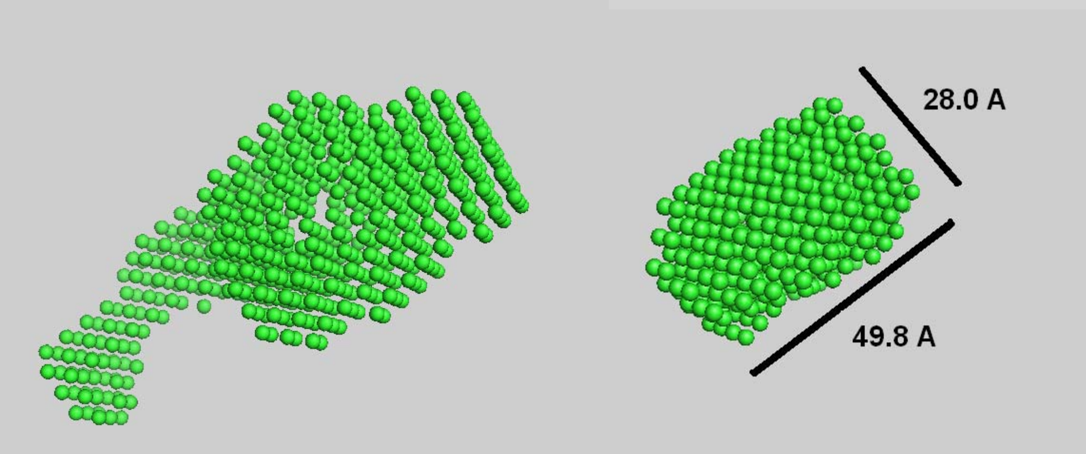
Fraction A8, 10% glycerol

5 mg/ml – spectrofotometer
1 mg/ml – SAXS



Model





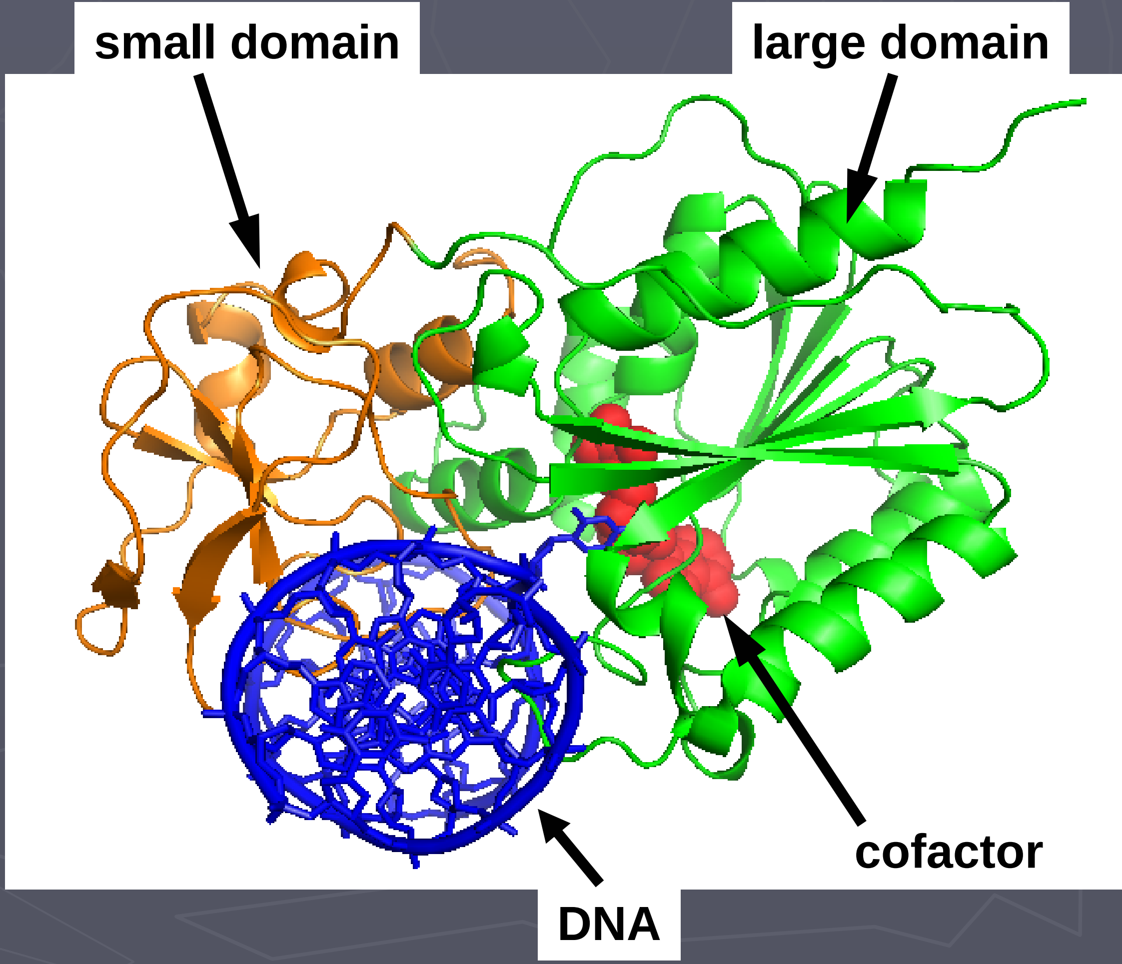
Radius of gyration	30.42
Maximum diameter	108.9
DAM shape anisometry	0.6274
DAM non-prolateness	0.0
DAM non-oblateness	0.4738

HhaI – well studied methyltransferase

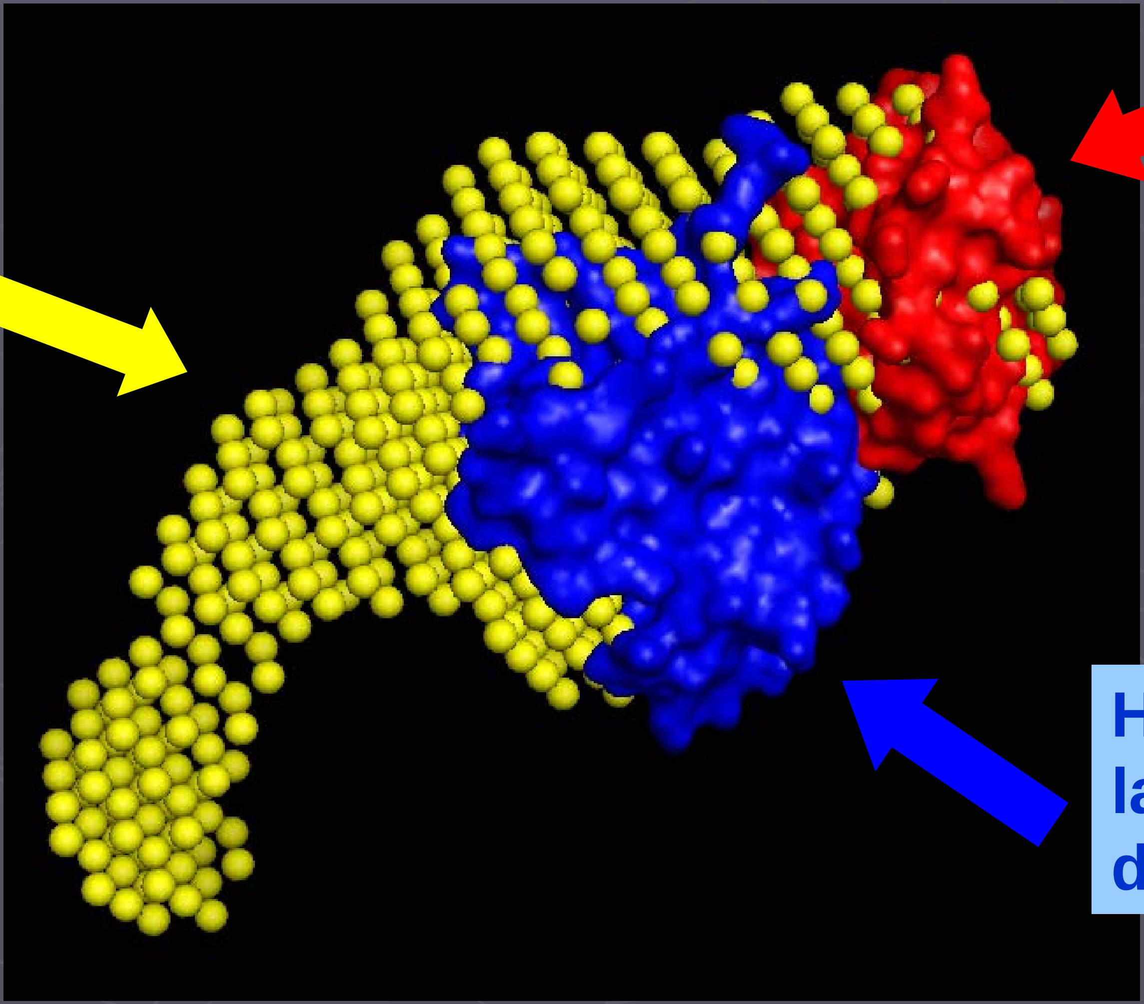
- *Haemophilus parahaemolyticus*
- 327 amino acid residues
- 37.0 kDa
- methylates the C5-atom of cytosine in the recognition sequence

5′ – GCGC – 3′
3′ – CGCG – 5′

- Monomer



Superposition



NlaX model

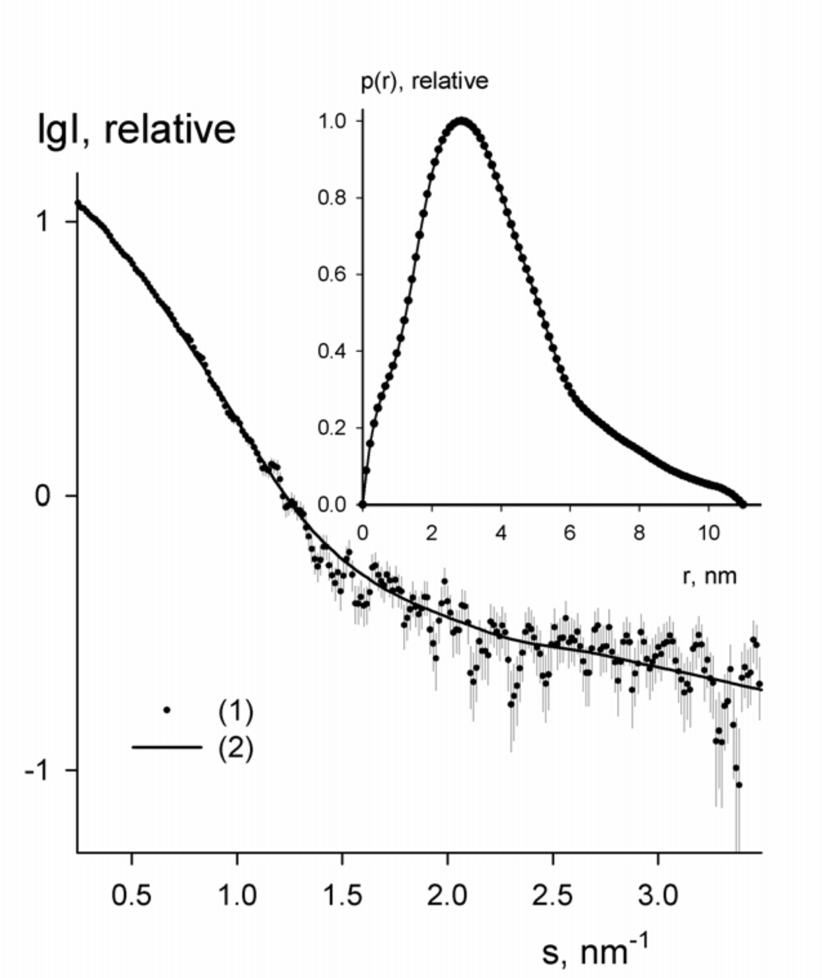
HhaI small domain

HhaI large domain

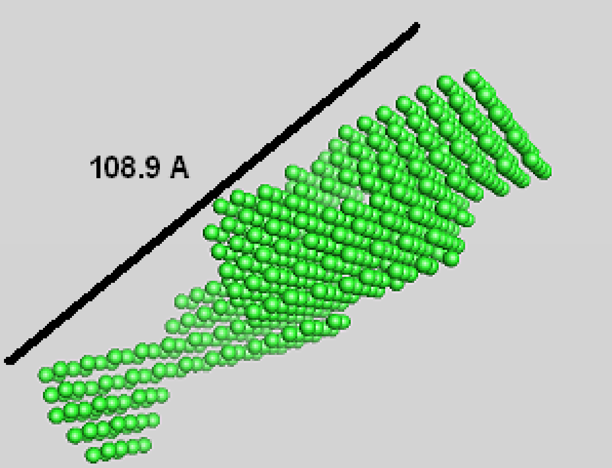
SAXS

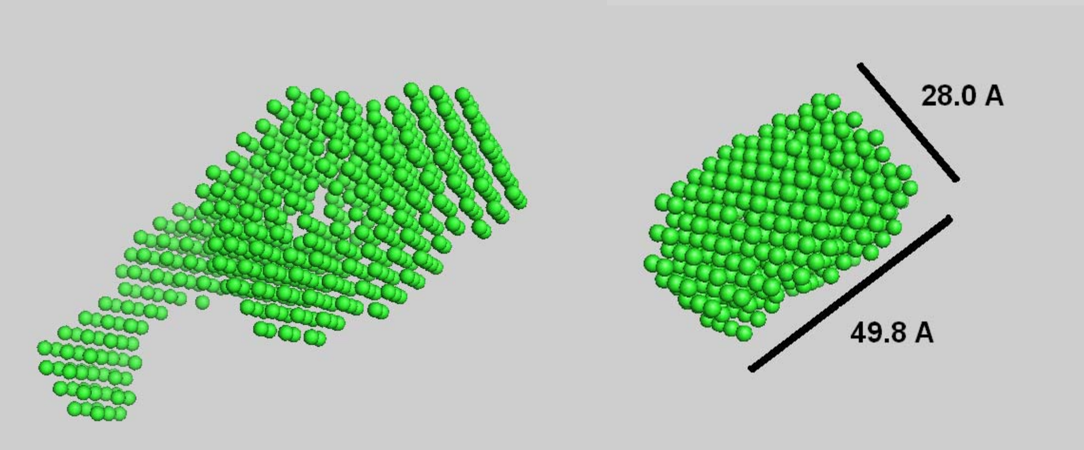
Fraction A8, 10% glycerol

5 mg/ml – spectrofotometer
1 mg/ml – SAXS



Model





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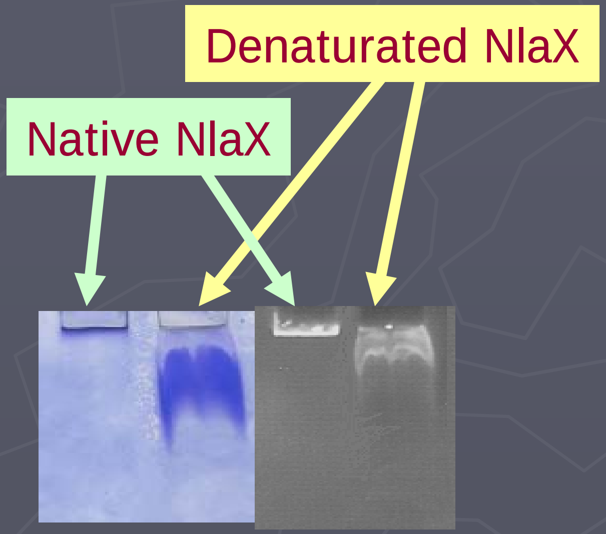
NlaX on native gels

- Native and denatured NlaX:

staining with EtBr and coomassie

DNA?



Denaturated NlaX

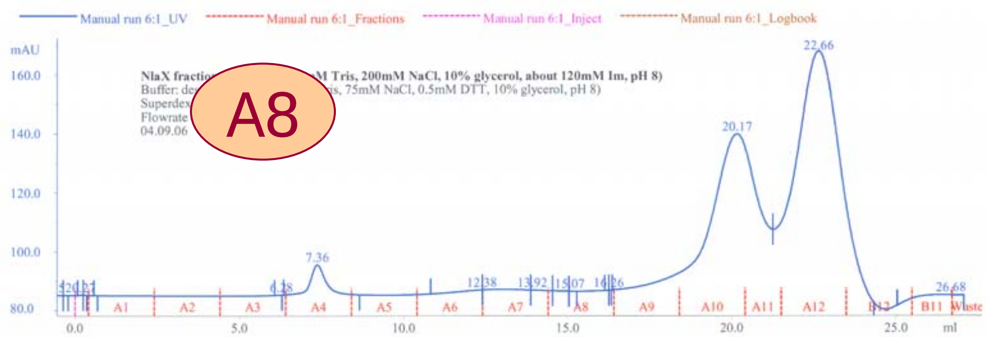
Native NlaX

Coomassie EtBr

Gel filtration

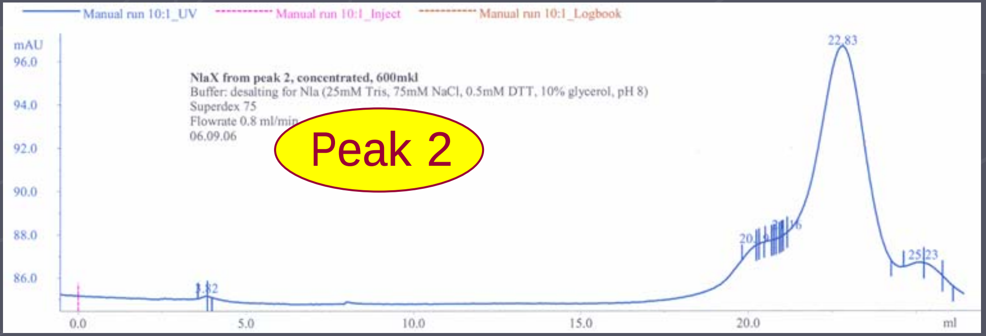
Superdex 75

- ► Both peaks contain NlaX
- ► No dynamic equlibration between peaks



A8

Peak 1



Peak 2

Acknowledgements

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