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# NMR quality control in fragment based screening

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- why NMR quality control ?
- automatic sample preparation and measurement
- examples (passed and rejected compounds)
- bottom line

# Why NMR QC ?

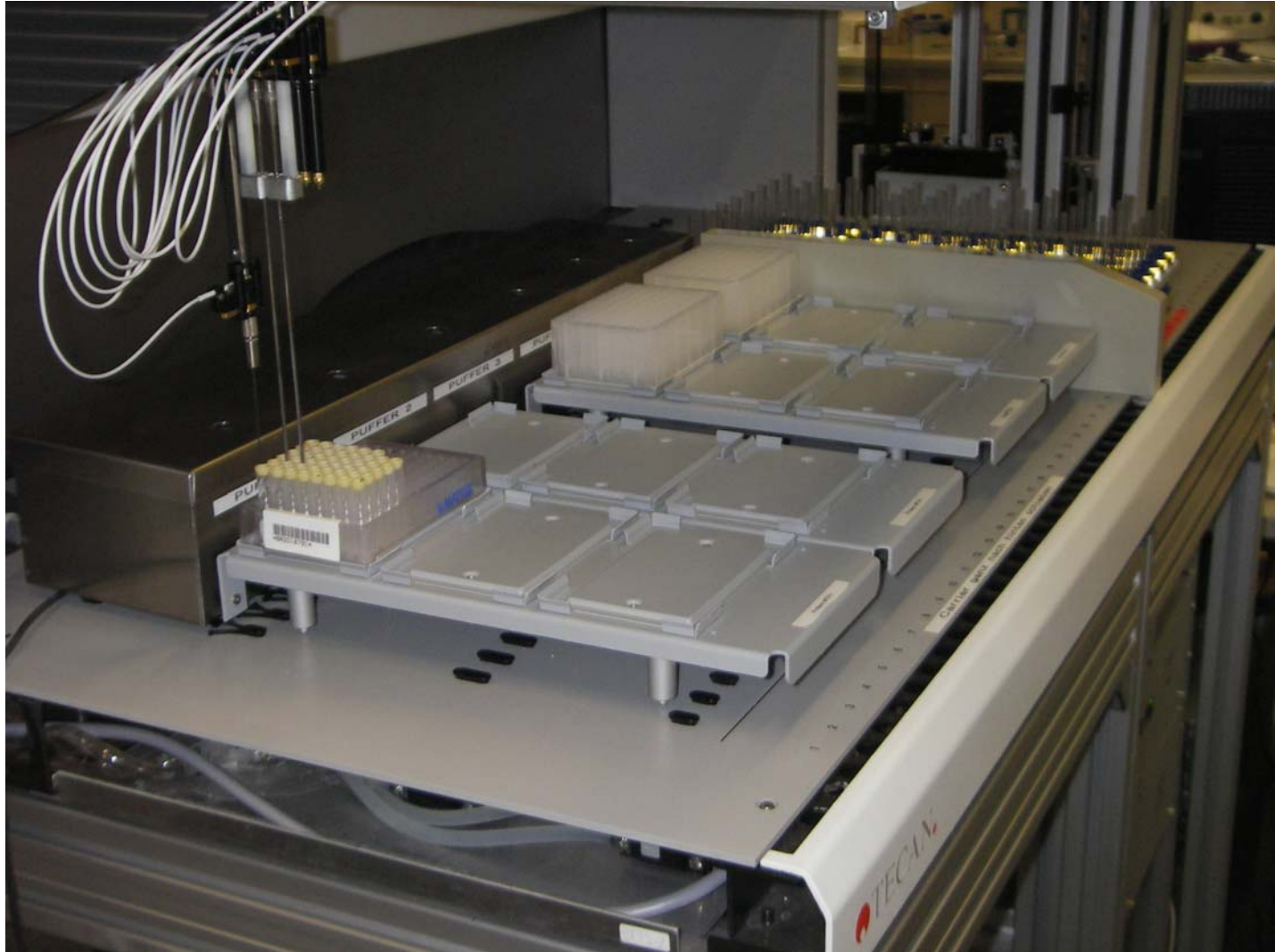
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- ligand based NMR screening methods rely on homogeneous solutions without micelles or solid particles (STD, WATERLOGSY,  $T1\rho/T2...$ )
  - no impure / unstable compounds
  - no poorly soluble compounds
  - no aggregating compounds
- holds true for other assays/methods in fragment-based screening and follow-up (functional assays, biophysical binding assays, XRAY/soaking)
  - false positives/negatives
  - promiscuous/overstoichiometric binding
  - nontypical binding curves
  - nontypical SAR

# NMR automation (Tecan robot)

pipetting DMSO stock solution from 96 well plate (Matrix tubes)

containers  
for buffer  
and protein  
solution  
(cooled)



NMR  
tubes

# NMR automation (Tecan robot)

mixing sample in 96 well deep well plate (MTP)

long needles

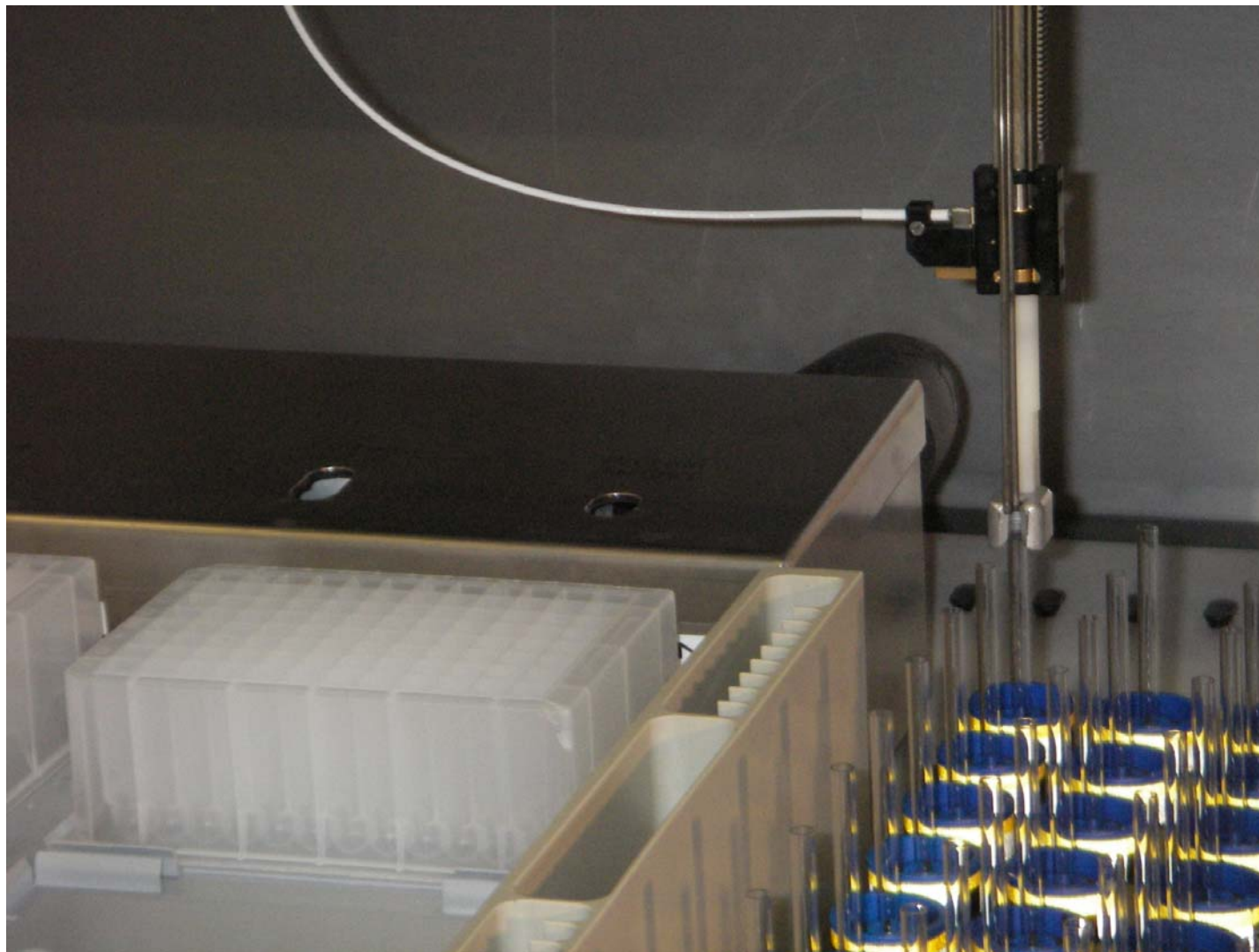
2mL MTP



NMR  
tubes

# NMR automation (Tecan robot)

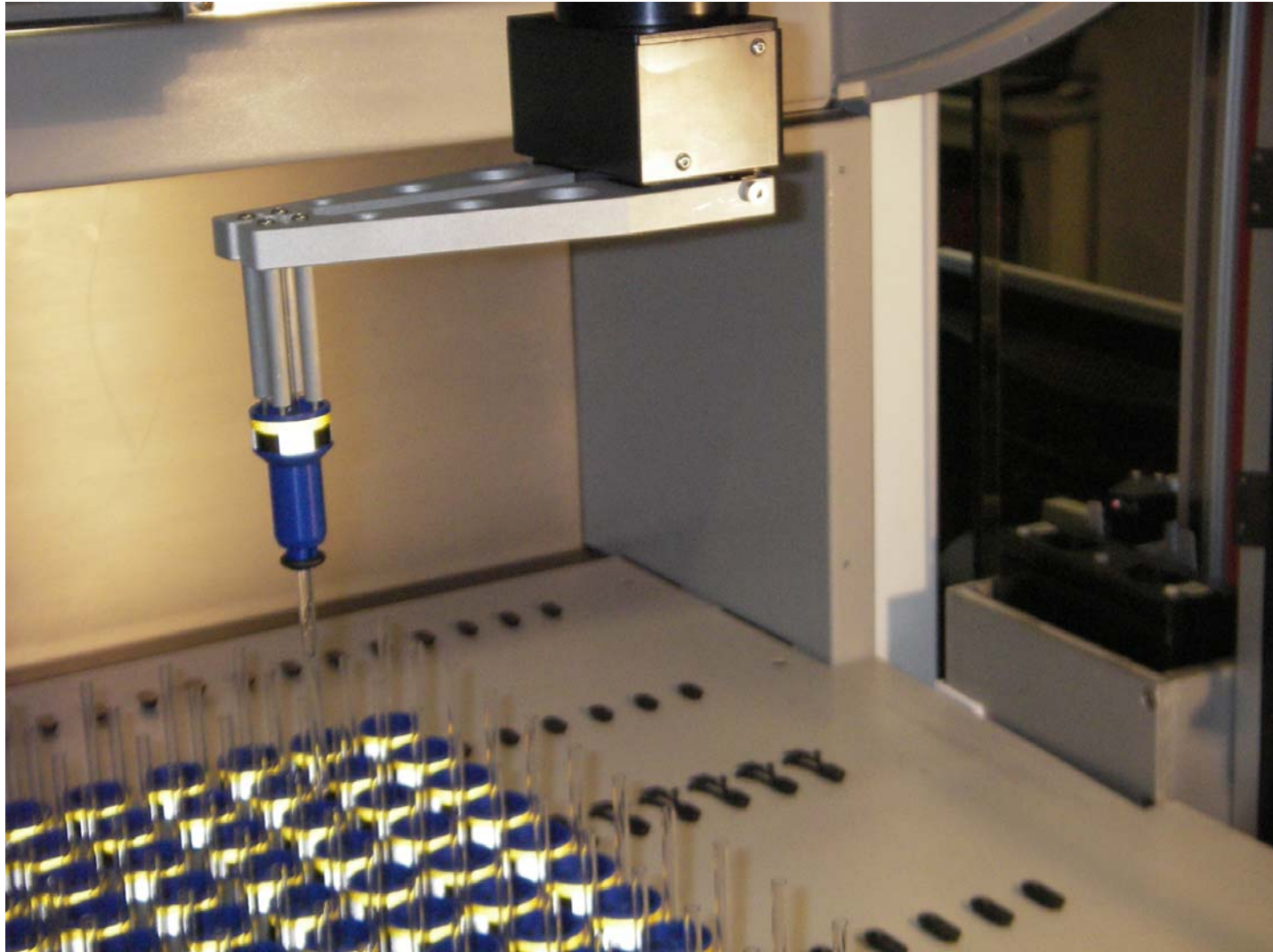
filling sample into NMR tube



# NMR automation (Tecan robot)

picking tube from rack

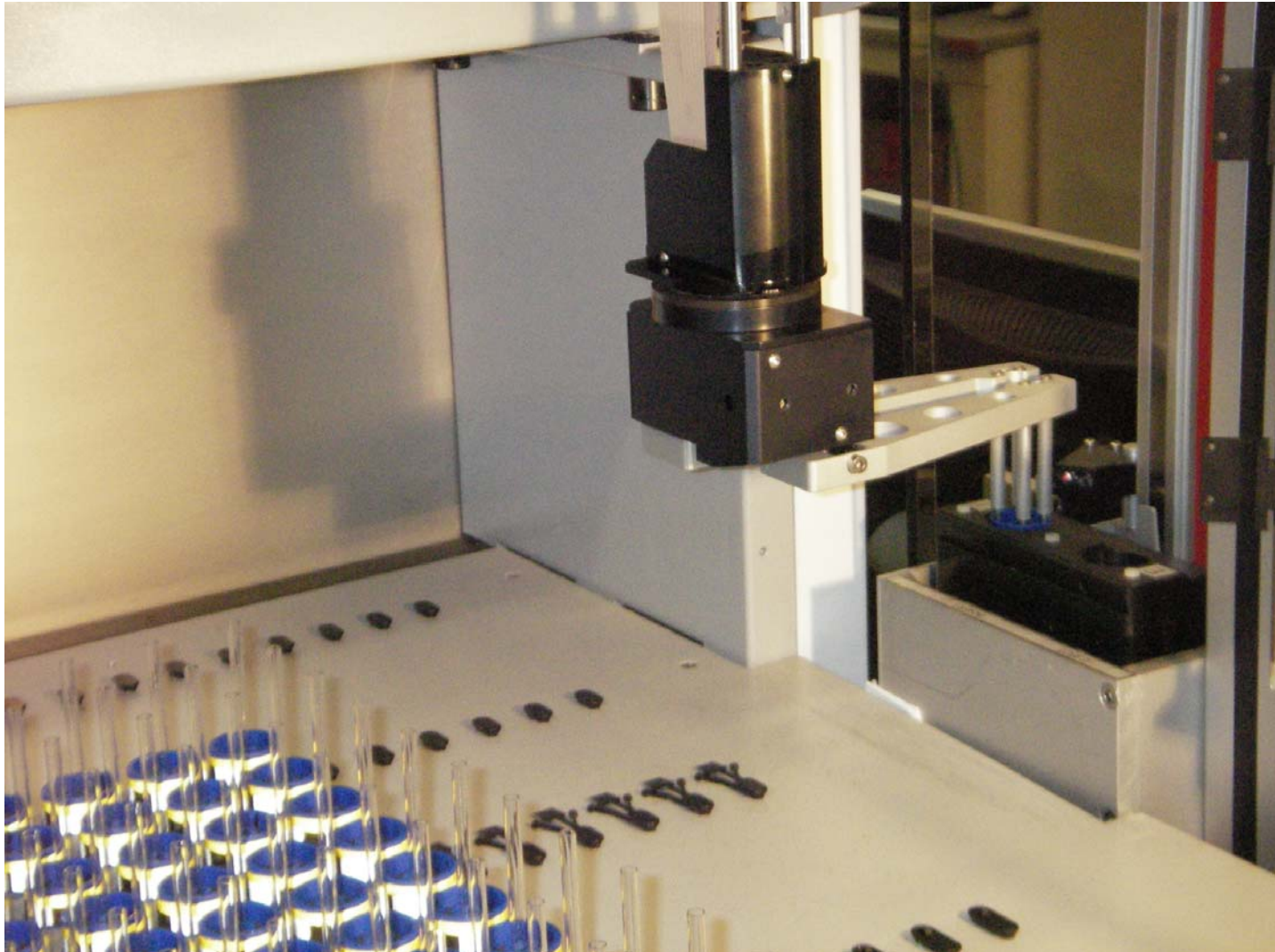
Tecan  
robotic arm



trolley  
(Bruker  
sample  
rail)

# NMR automation (Tecan robot)

placing tube into trolley





# NMR automation (Bruker sample rail)

transfer to magnet



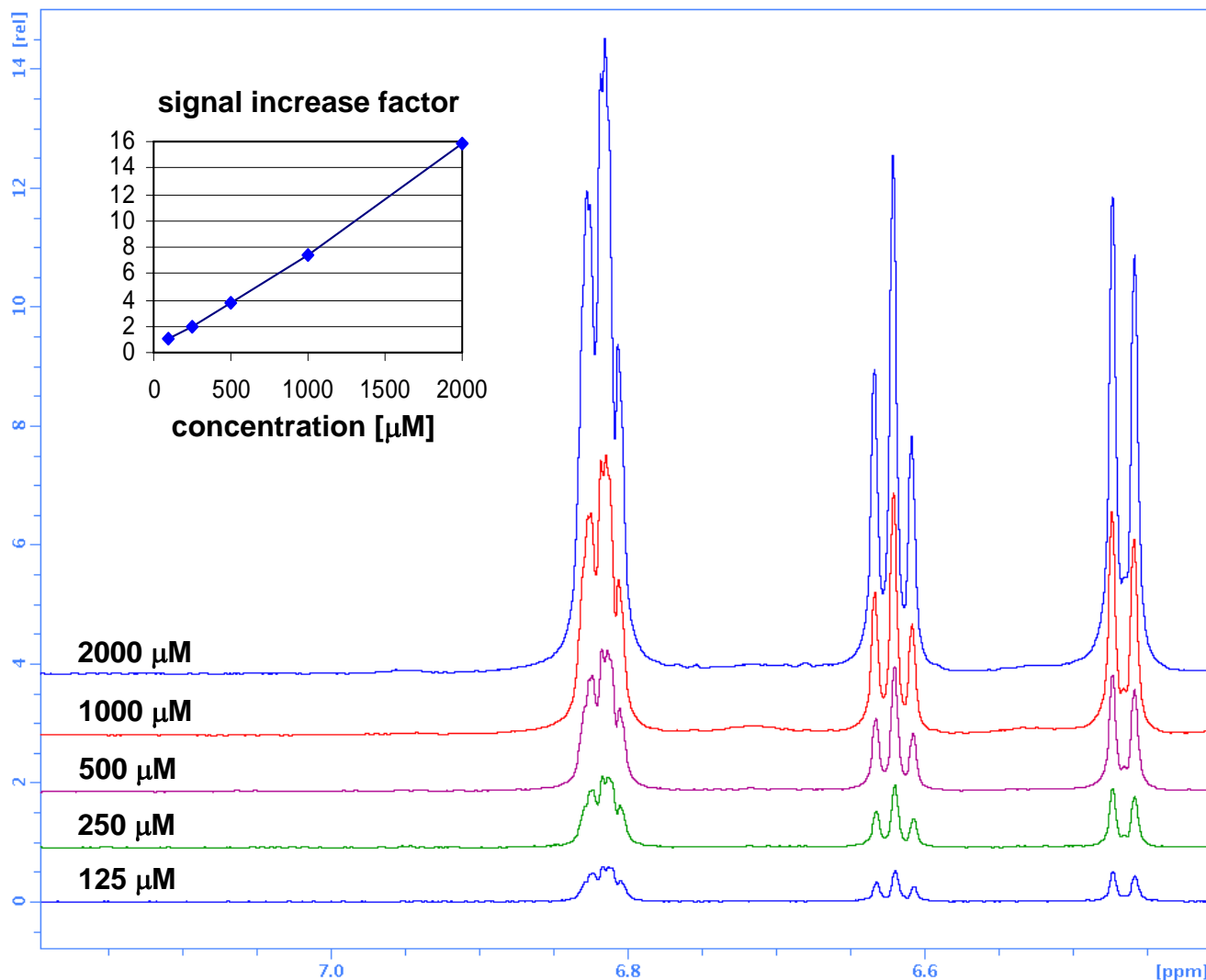
Bruker  
sample  
rail

Tecan  
robot

magnet

# NMR solubility in phosphate buffer pH 7.4

well soluble compound (2000  $\mu\text{M}$ )



## Solubility

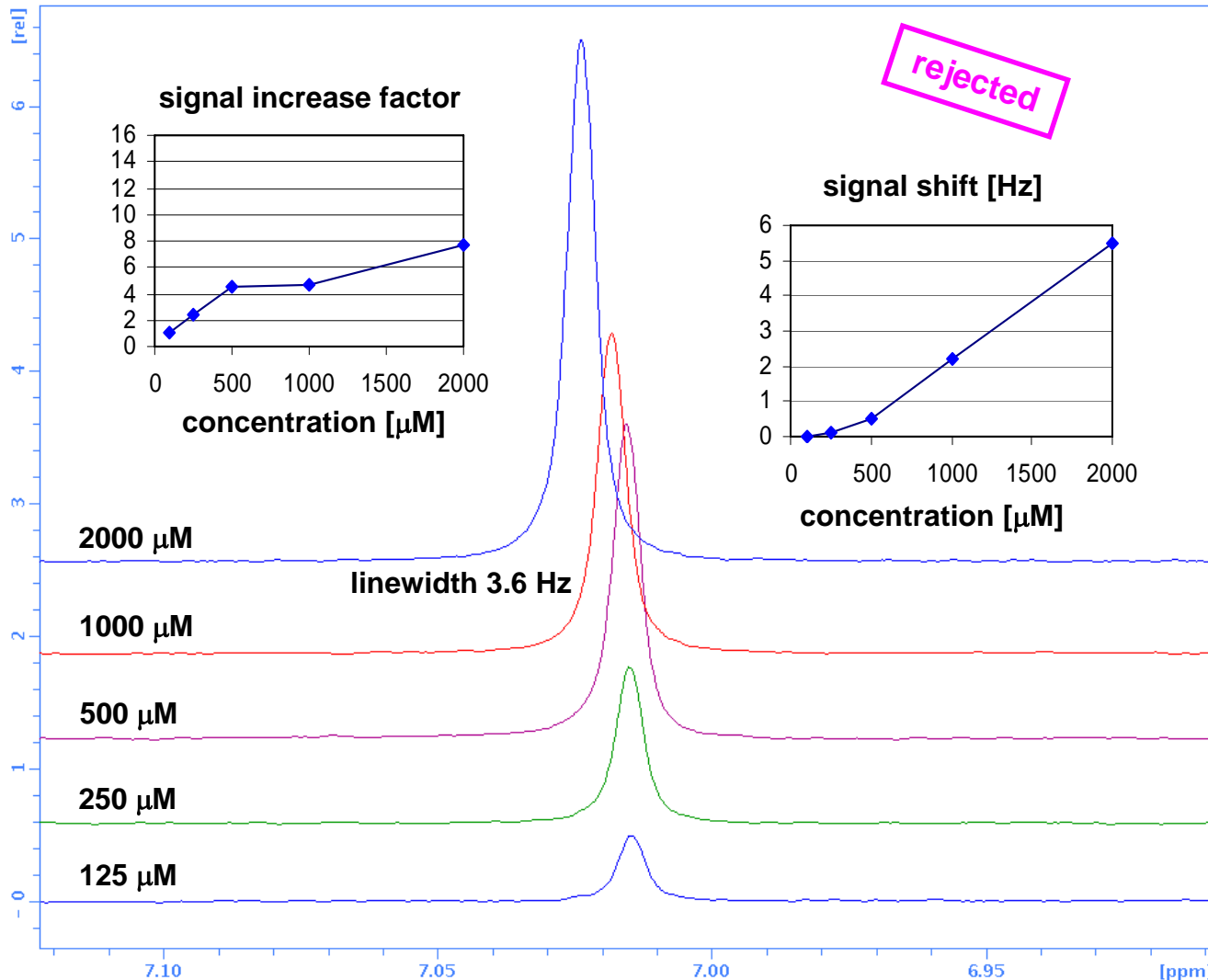
- 50 mM stock in  $\text{d}_6$ -DMSO
- 100 mM  $\text{PO}_4$  buffer, pH 7.4
- five concentrations: 125  $\mu\text{M}$ , 250  $\mu\text{M}$ , 500  $\mu\text{M}$ , 1000  $\mu\text{M}$ , 2000  $\mu\text{M}$
- measure only relative signal increase (scaling factor)
- no proton assignment
- no content determination

## Aggregation

- STD NMR spectrum @500  $\mu\text{M}$
- fast exchange in micelles

# NMR solubility in phosphate buffer pH 7.4

compound with restricted solubility (500  $\mu\text{M}$ )



## Solubility

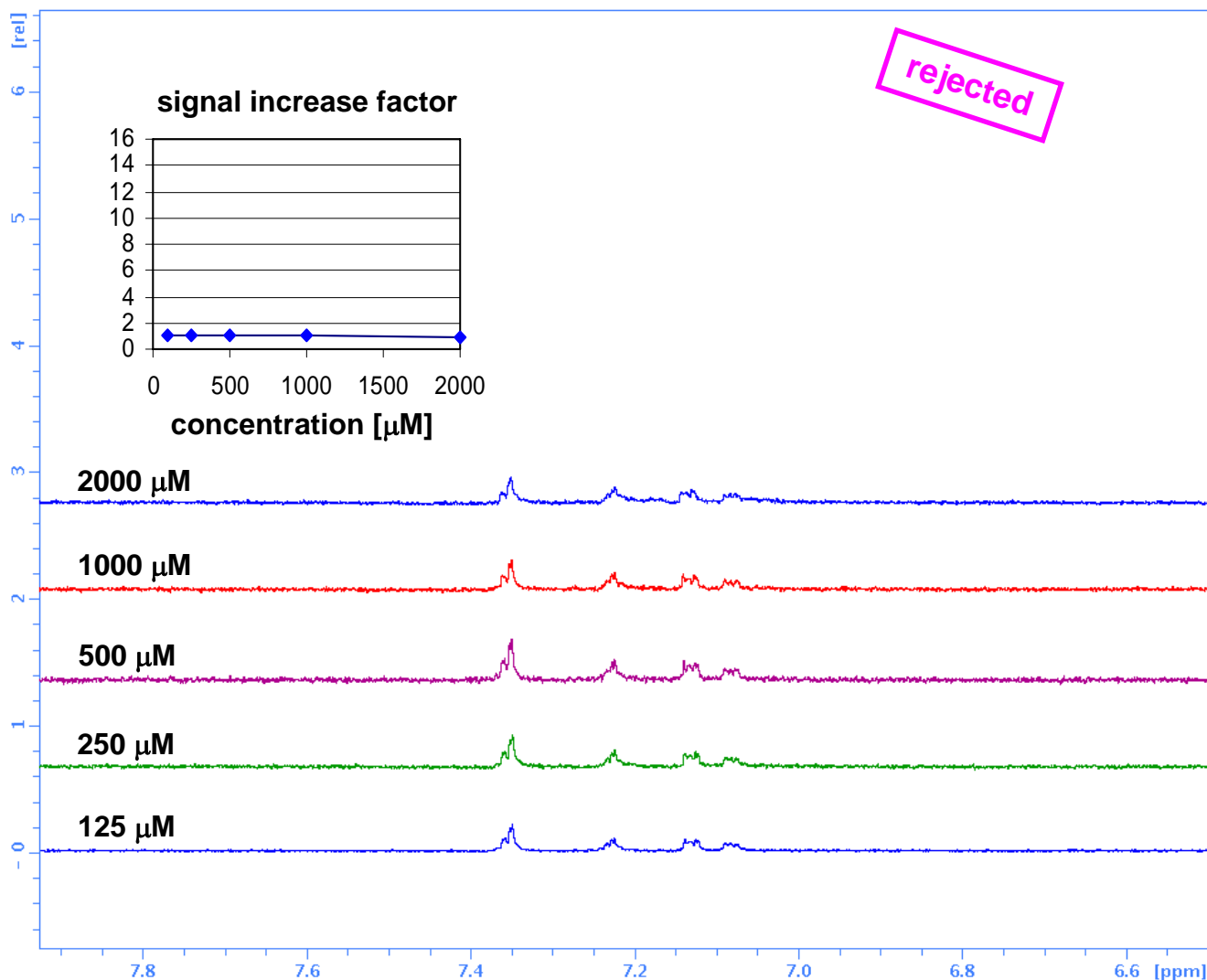
- up to 500  $\mu\text{M}$  in buffer (precipitation)
- rejected for XRAY (soaking)
- rejected for titration ( $K_D$ )
- might work for NMR@ < 250  $\mu\text{M}$

## No aggregation

- no STD NMR signals @500  $\mu\text{M}$  (@1000  $\mu\text{M}$ ?)
- no line broadening
- downfield shift 5 Hz (kinky)

# NMR solubility in phosphate buffer pH 7.4

poorly soluble compound ( $< 125 \mu\text{M}$ )



## Solubility

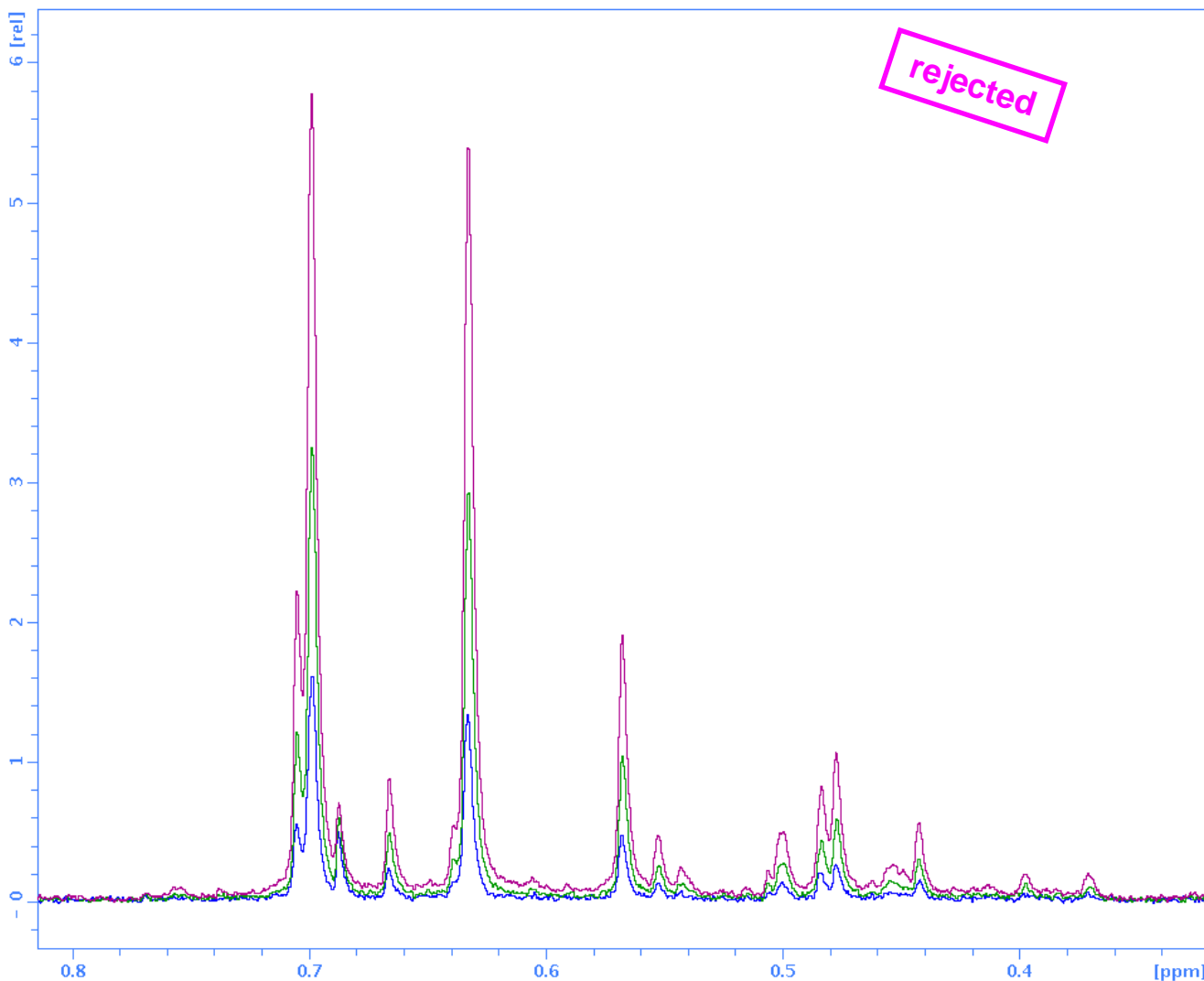
- solubility  $< 125 \mu\text{M}$

## No aggregation

- no broadening, no shift
- no STD NMR signals @500  $\mu\text{M}$
- solids in slow exchange

# NMR solubility in phosphate buffer pH 7.4

mixture / decomposition

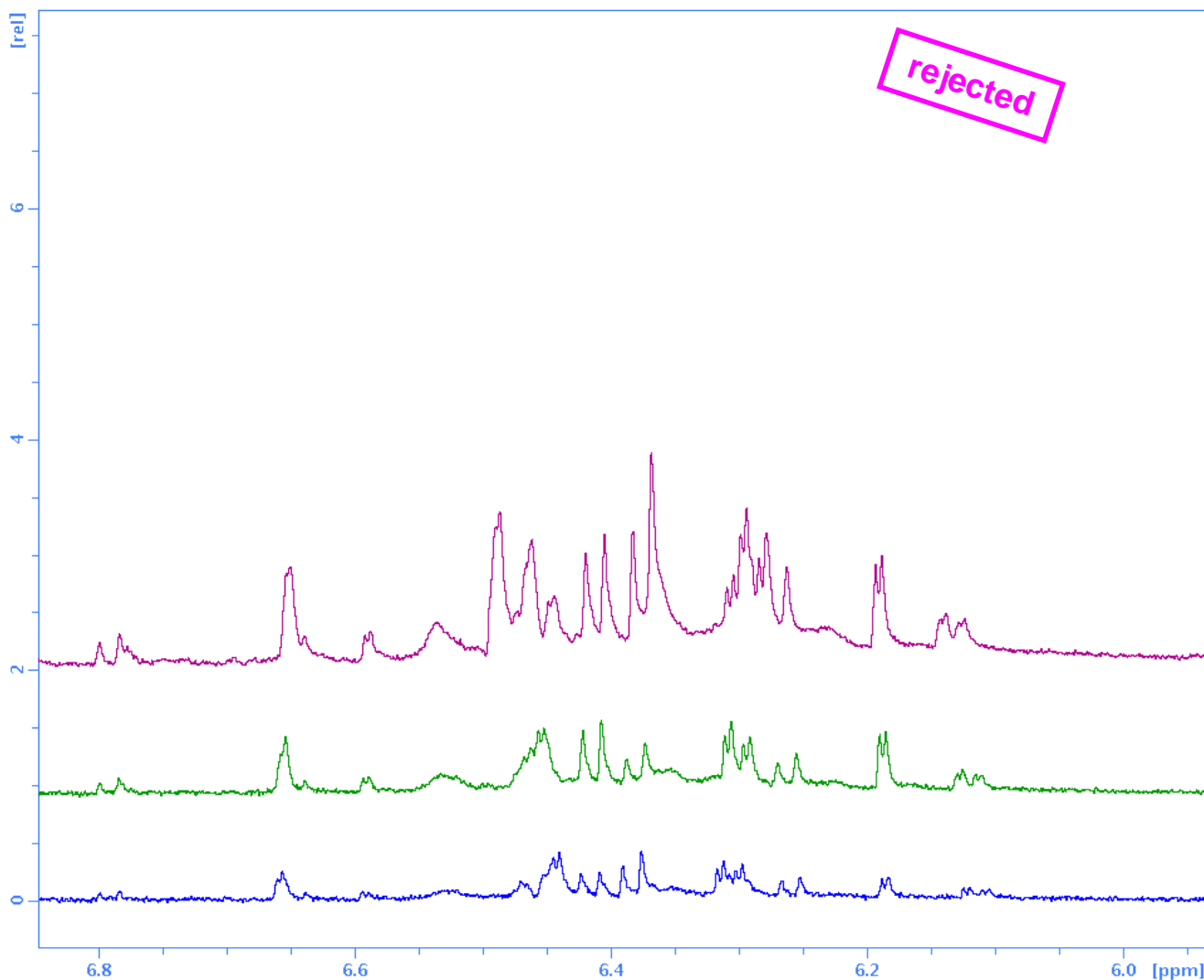


## Purity

- expected:  
2 methyl signals

# NMR solubility in phosphate buffer pH 7.4

mixture / decomposition

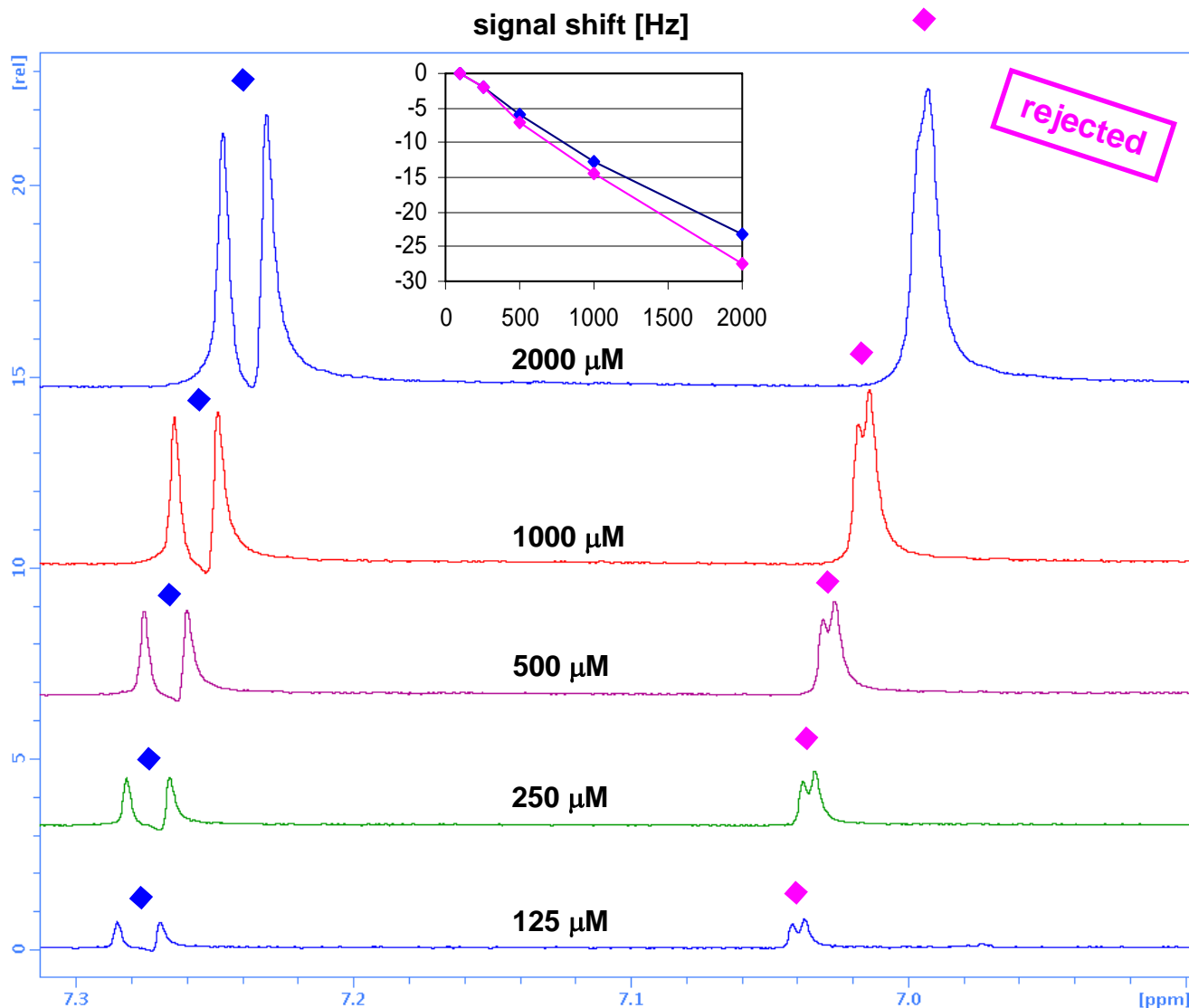


## Purity

- expected:  
3 aromatic signals

# NMR solubility in phosphate buffer pH 7.4

## aggregation



## Solubility

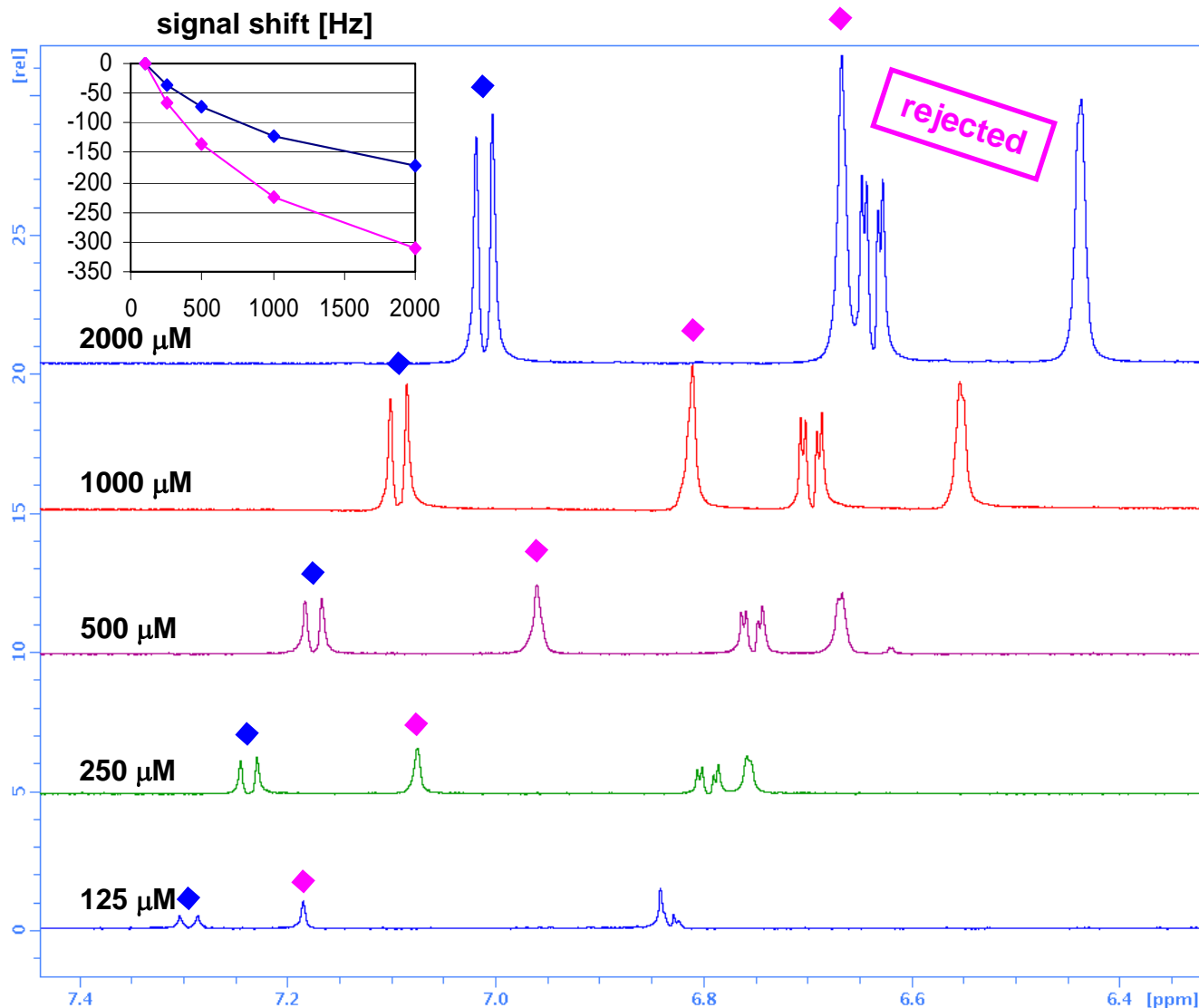
- solubility up to 2000  $\mu\text{M}$  from relative signal increase (scaling factor)

## Aggregation

- STD NMR signals @500  $\mu\text{M}$ : 16 %
- linear signal shift @2000  $\mu\text{M}$ : > 25 Hz (CSA/ringcurrent effect in micelles or clusters)

# NMR solubility in phosphate buffer pH 7.4

## aggregation



## Solubility

- solubility up to 2000  $\mu\text{M}$  from relative signal increase (scaling factor)

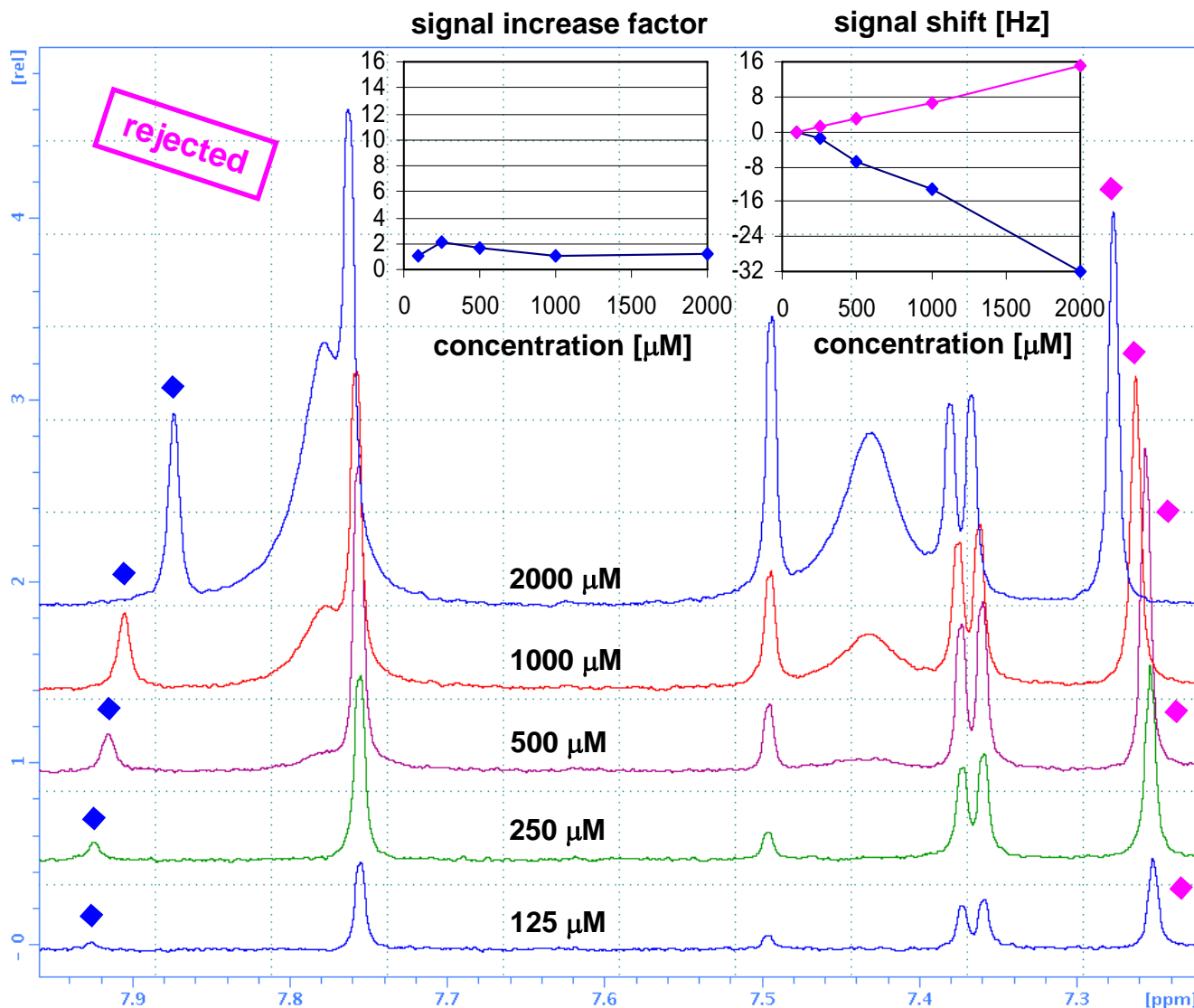
## Aggregation

- no STD NMR signals @500  $\mu\text{M}$
- downfield shift 150-300 Hz (CSA/ringcurrent effect)



# NMR solubility in phosphate buffer pH 7.4

aggregation



## Solubility

- solubility up to 250  $\mu\text{M}$  from relative signal increase (scaling factor)

## Aggregation!

- STD NMR signals @500  $\mu\text{M}$ : 17-63 %
- broadening in aggregates @500  $\mu\text{M}$
- downfield and upfield shift (CMC?)

# NMR QC

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## Bottom line

- there is whole universe between perfect solution and solid precipitate  
(with WATERLOGSY and  $T1\rho$  experiments it might even grow)
- highly dynamic aggregates only show STD effects
- slower exchange leads to signal broadening
- some compounds show shifts only (solvation properties)
- we won't notice a 50 % content  
(trifluoroacetate MW+113, tosylate MW+171)
- proton assignment / content determination is desirable (concentration standard)