Practical Considerations for Enlightened Compound Management

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LRIG Mid Atlantic
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Nexus Biosystems

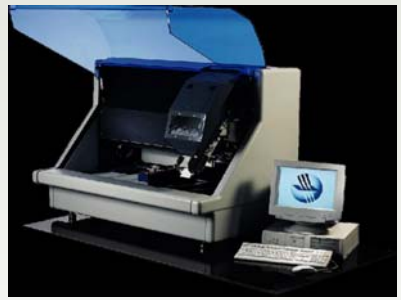
- **IRORI → Discovery Partners → Nexus Biosystems**
- **Providing drug discovery technology platforms for ~ 10 years!**

NEXUS Technology Platforms

Combinatorial Chemistry Automation

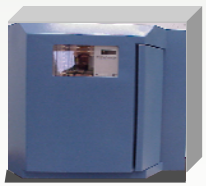
IRORI™ ***RF MicroKan Synthesis*** **Products**

IRORI™ ***2D NanoKan & X-Kan Synthesis*** **Products**



Protein Crystallography

Crystal Farm™ ***Automated Crystal Incubation & Imaging***



Compound Storage & Retrieval

The Universal Store ***Automated –20C, N2, with High-Speed Cherry Picking***

The Universal Store

- **Flexible Automation**
  - Store tubes, vials, bottles, microplates, etc.
  - Store at –20°C, nitrogen
  - High speed loading & retrieval
  - Output defrosting

- **Configurable Customization**
  - 10,000 to 100 million samples
  - High speed cherry picking options
    - Up to 100,000 compounds picked per day
    - Picking at –20°C, nitrogen atmosphere

- **Installations include Sanofi-Aventis and the NIH Small Molecule Repository**

Compound Stability

- **Ideal**
  - Cold, inert, dry
  - Solid phase
    - Dry film, powder
  - Compound transfer is slow
    - Weighing
    - Volatile solvent transfer
  - Apply to long term storage of compound reserve
- **Practical**
  - DMSO solutions are a compromise
    - Fast compound transfer
    - Short term (“working”) storage
  - *Keep the water out!*

JIT Working Store

- **Individual tubes replace plates for storage**
  - Secure, individually identified
- **Cherry pick mechanically vs. liquid handling**
  - Avoid freeze-thaw of non-selected samples
  - Minimize exposure to water & oxygen
- **No longer limited by slow liquid cherry picking!**
  - **Up to 100,000 per day**
  - Hit follow-up
  - EC50s
  - Selective JIT primary screening sets
  - Iterative screening

Working Store Temperature

- **Colder is better**
  - Drier
    - 50% RH @ 25 ºC → 12 g/m3
    - 100% RH @ -20 ºC → 1 g/m3
  - More stable
- **Practical temperature**
  - Depends on time in storage vs. time out of storage
  - Example:
    - Working store temp. = –20 °C
    - Samples removed from storage for 1 day at a time, 20 times during year
    - Degradation rate ~ 2x per 10 °C → 23x at 25 °C vs. –20 °C
    - Degradation 20 days at 25 °C > Degradation 345 days at –20 °C

Working Store Compound Concentration
(in DMSO)

- **In practice: 1 – 30 mM**

- **Lower is better (for compound management)**
  - Solubility
    - 16% not soluble at 10 mM
    - 65,500 member drug-like compound set*
  - Liquid handling easier (µL instead of nL)

- **Higher is better (for biology)**
  - Minimize DMSO at screening concentrations (1 µM – 10 µM)

- **Compromise at ~ 1 mM**
  - 1 mM → 10 µM, 1% DMSO, or
  - → 1 µM, 0.1% DMSO, or
  - → 50 µM, 5% DMSO

*Balakin et al, J. Biol. Screen. 9(1); 2004

Screening Concentration

- **In practice: 1 – 10 µM**

- **Solubility**
  - Amphora Discovery*
    - ~75% of compounds below expected 50 µM
    - ~55% of compounds below 10 µM
    - Buffer with 5% DMSO
    - 2,000 member diversity library
  - Pfizer**
    - ~95% of compounds below expected 200 µM
    - ~45% of compounds 25 µM – 100 µM
    - ~50% of compounds below 25 µM
    - Water with 5% DMSO
    - 1,000 member diverse set

*Popa-Burke et al, Anal. Chem. 76(24); 2004

**Nakayama, San Diego HTS Discussion Group, Dec. 7, 2005

Screening Plates

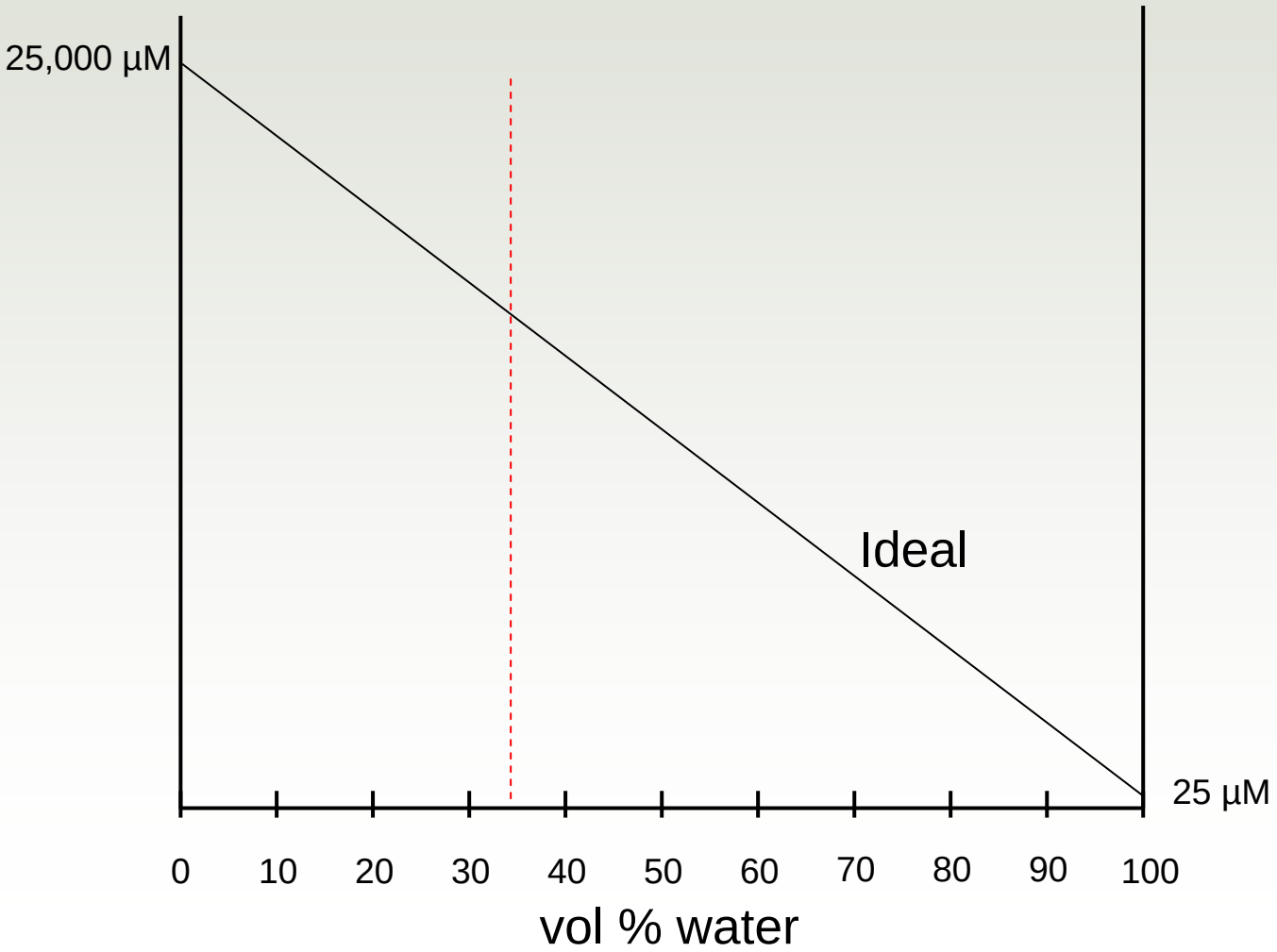
- **Many compounds not soluble at 10 µM!**
  - Best would be to measure concentration
    - Not practical for HTS
  - Anticipate insoluble compound

- **Deliver compounds in DMSO or dry?**
  - In DMSO
    - Advantage:
      - Rapid mixing
    - Disadvantage
      - Crashes out of solution unpredictably
  - Dry
    - Disadvantage
      - Slow dissolution or good mixing (sonication) needed
    - Advantages:
      - Assay can dictate % DMSO (if any) ← **Better for biology**
      - Insoluble compound stays on well surface ← **Better for biology**
      - Easy to transport (dry) ← **Better for compound management**
      - Working Store concentration can be low! ← **Better for compound management**

Reasons for Lack of Compound
(at screening)

- **Insoluble in working store DMSO**
  - Working store concentration too high
  - Precipitation as water is absorbed
- **Insoluble in aqueous buffer**
- **Precipitation during intermediate dilutions**

Compound Solubility
In DMSO/Water



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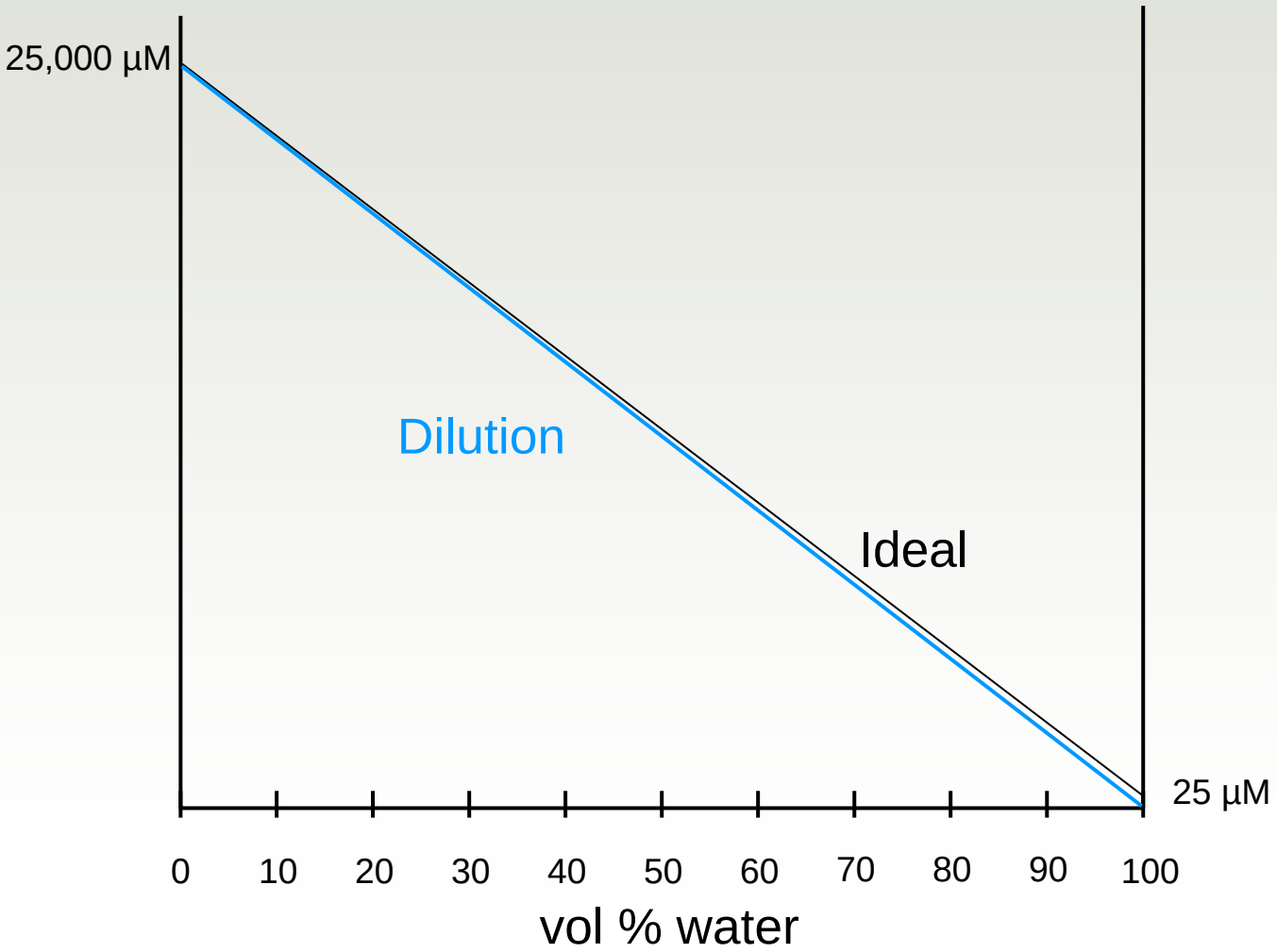

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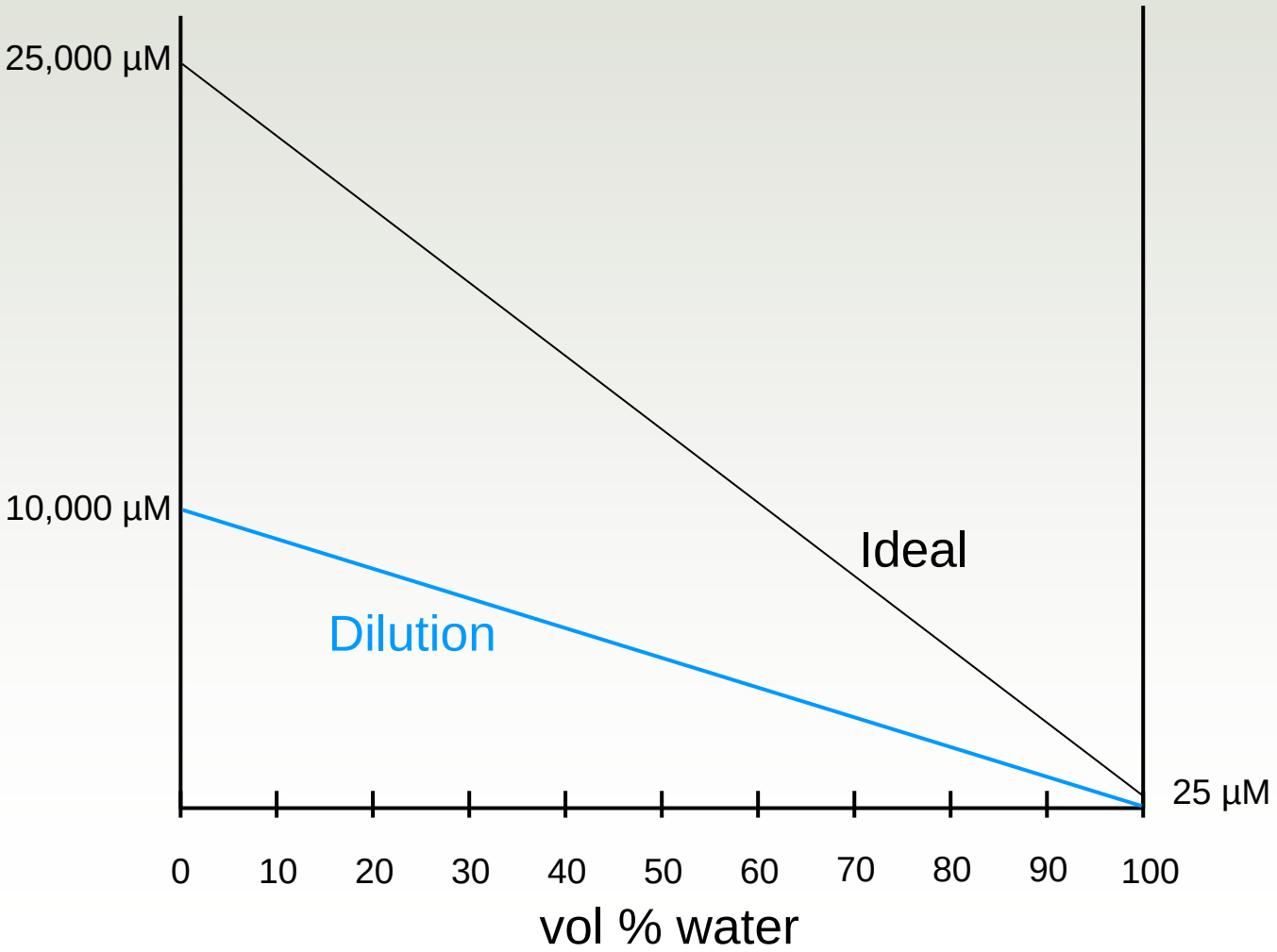
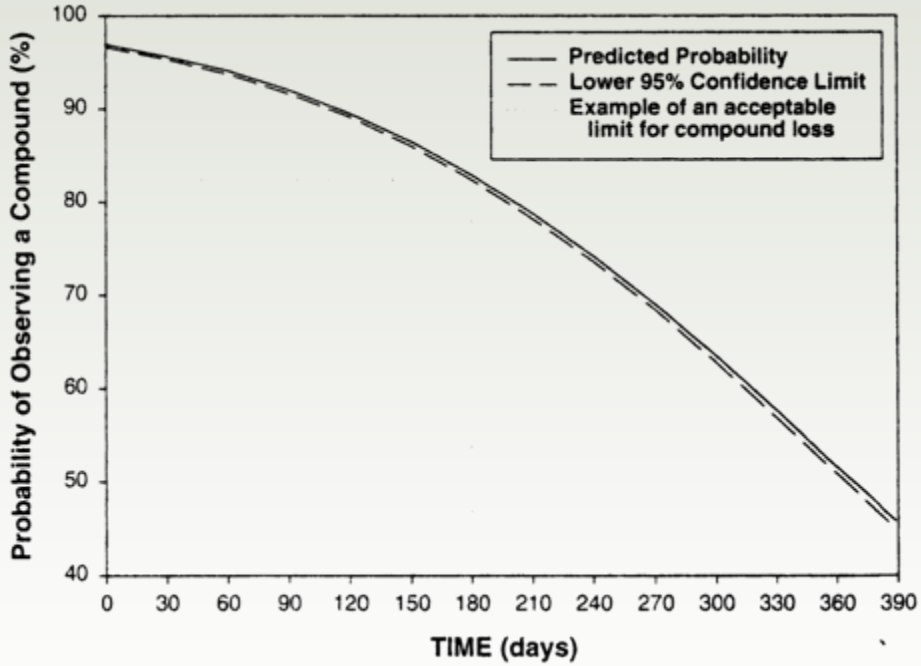


Literature Data #1

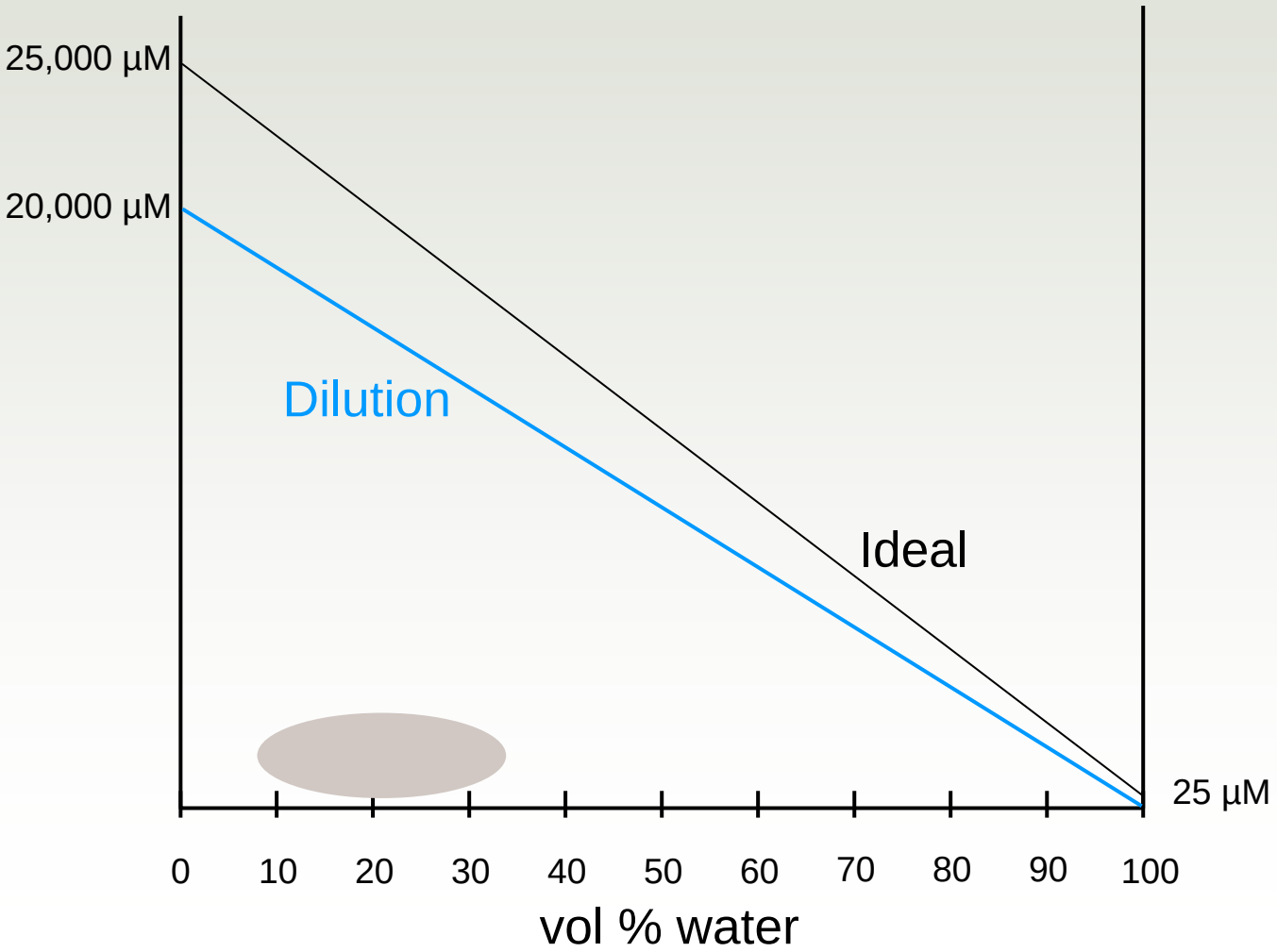
- **Experiment at P & G***
  - 7200 compounds
  - 20 mM in DMSO
  - RT, ambient air
  - 96-well plates with capmats

- **Results**
  - 50% “loss” after 1 year
  - Probably precipitation due to water absorption



*Kozikowski et al, J. Biol. Screen. 8(2); 2003

Compound Solubility
in DMSO/Water



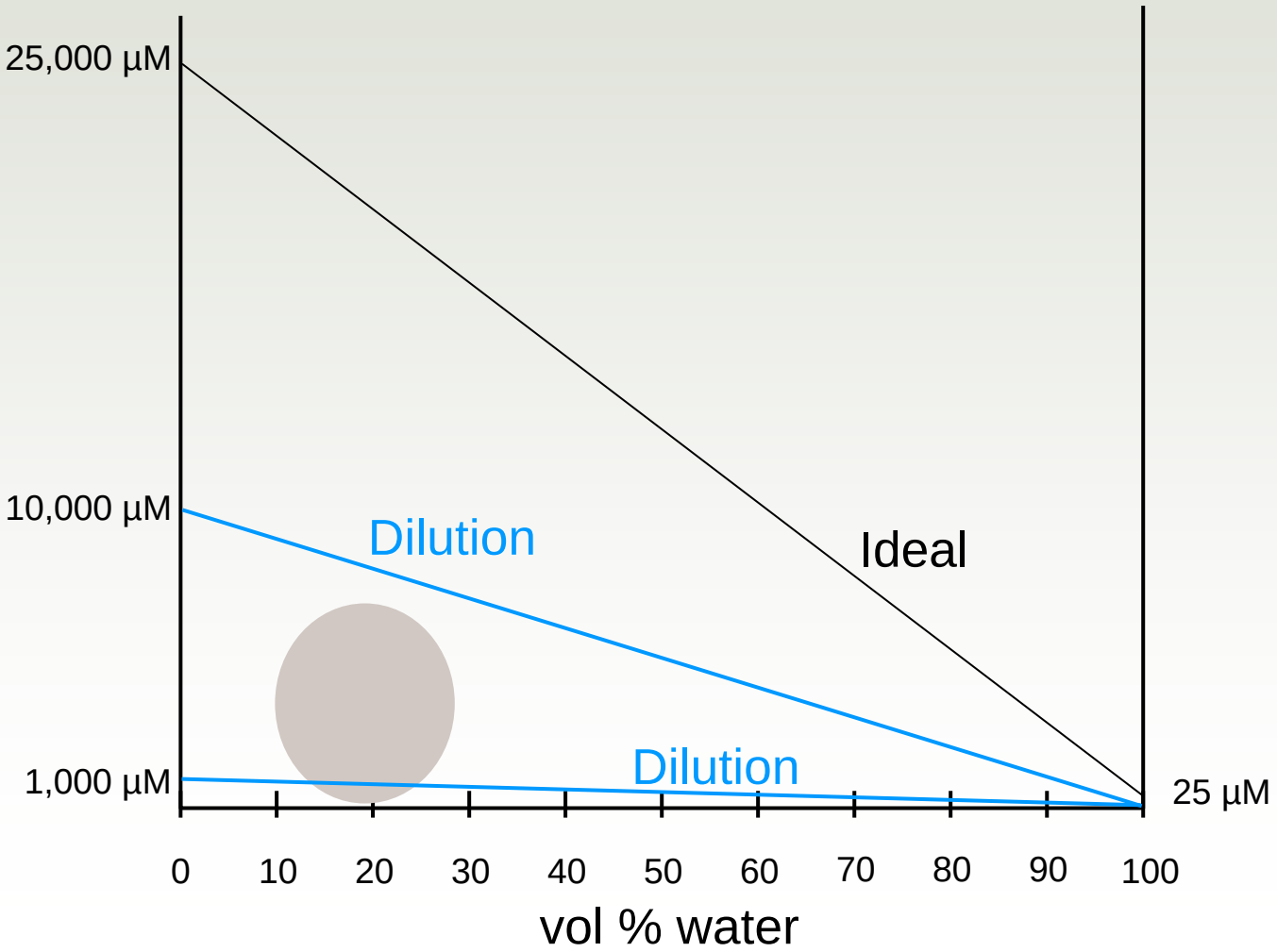
Literature Data #2

- **Experiment at NCI***
  - 1990 compounds
  - 10 mM in DMSO
  - Frozen for 1 year
  - 96-well plates with heat seals

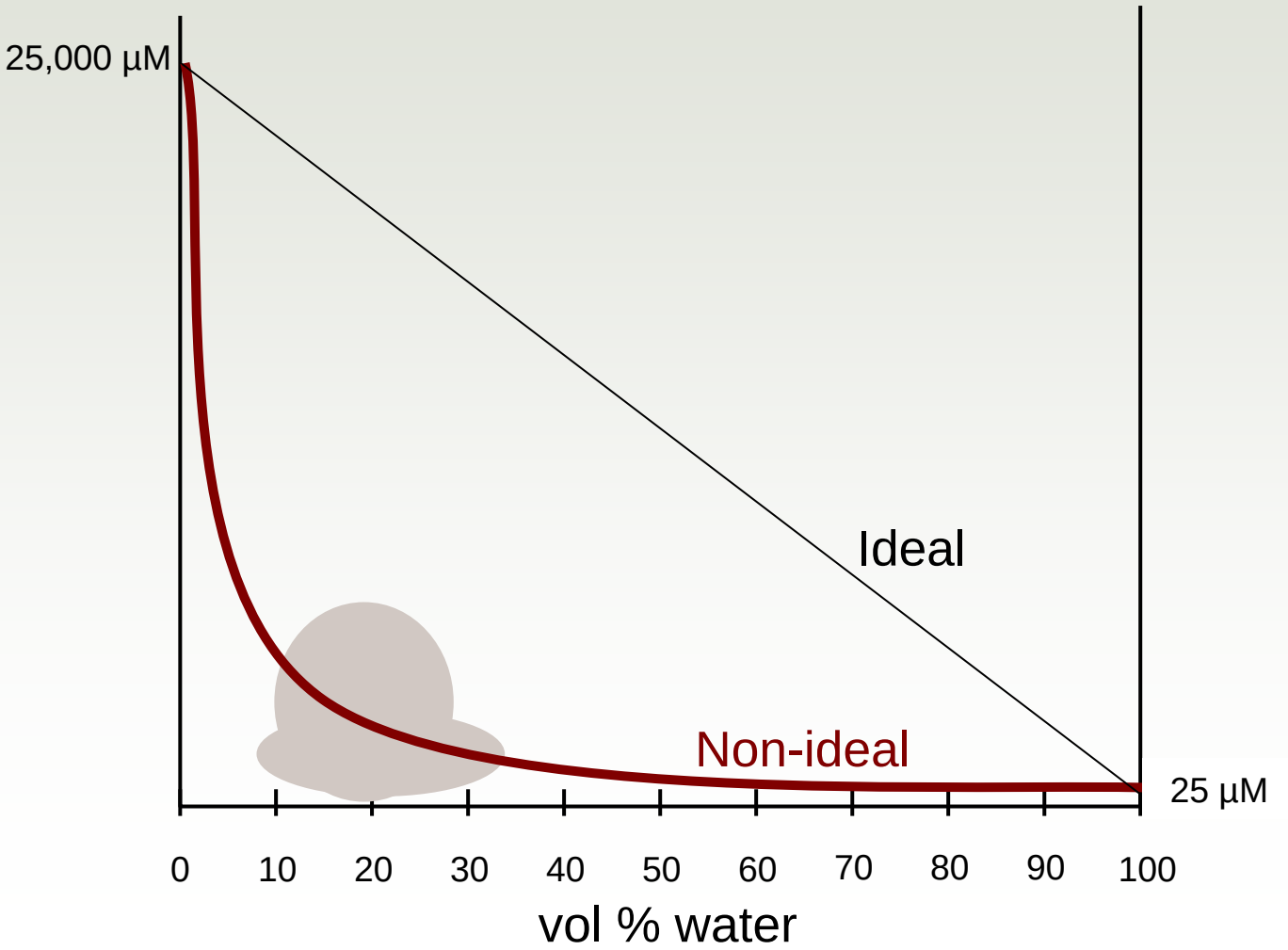
- **Results**
  - Precipitation of 20% of compounds
  - Compound integrity confirmed by drying and re-solubilizing in DMSO
  - Supernatent concentrations of 1 µM – 7 mM
  - No precipitation observed when 1 mM solutions stored!

*Waybright et al , Overcoming the Problems Associated with Long-Term Storage of Compounds in DMSO. Presented at 2004 NCI-Frederick/Ft. Detrick Spring Research Festival, May 12-13, 2004 Ft. Detrick, Maryland

Compound Solubility
in DMSO/Water



Compound Solubility
in DMSO/Water



Compound Solubility
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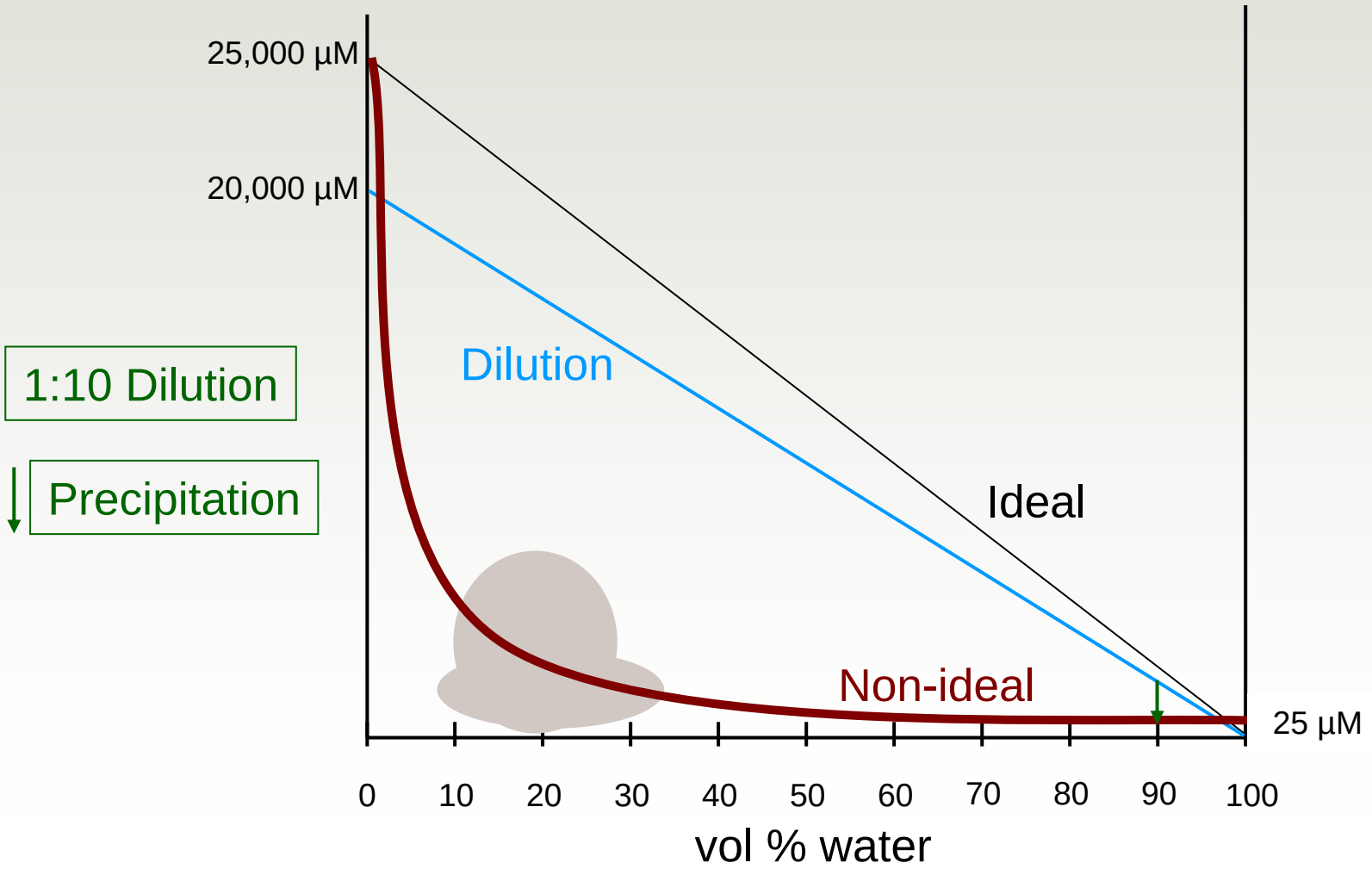

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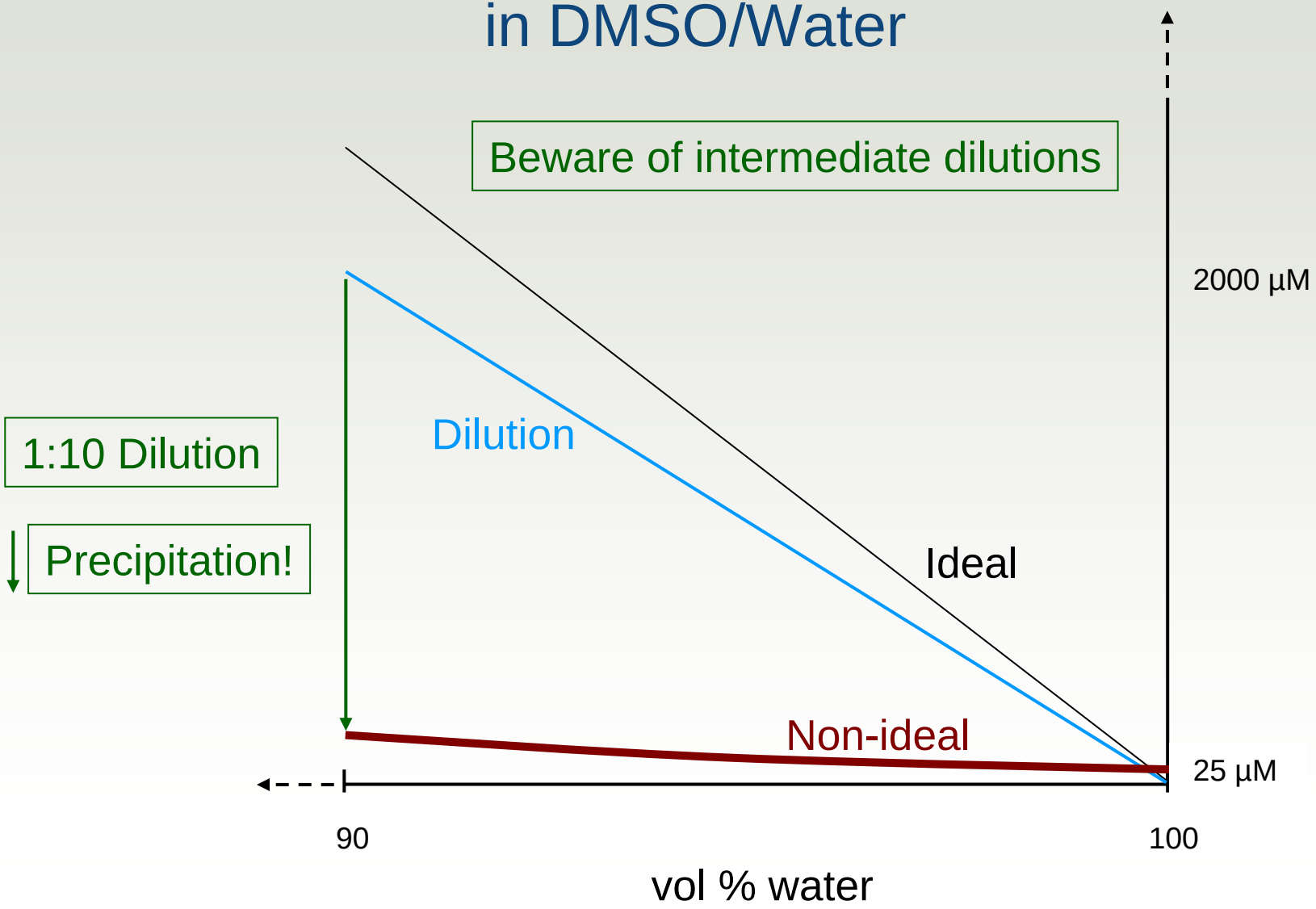


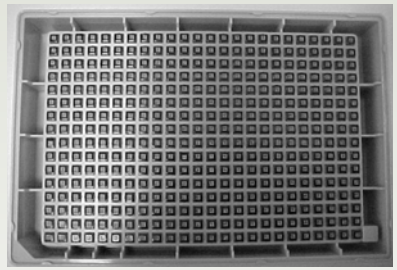
Summary of DMSO Concentrations

- **Consider lower storage concentrations (~1mM)**
- **Avoid intermediate dilutions**
- **Consider delivering dry compound into final screening plates**

Working Store

Multiple copies vs. Multiple access

- **Multiple single-use copies**
  - Pros
    - Saves washing steps during liquid handling
    - Handle each copy only once
      - Less care needed during handling
  - Cons
    - Inflexible
      - Pre-determined amount of compound, number of copies
    - Must know process and capacity needs in advance
    - Wasteful of compound and storage space (unused copies)
    - Disposable costs
  - Best case:
    - Store dry, add final buffer directly to tubes
    - (then mix and transfer to assay plates)



384 tube rack
with barcodes

JIT Plate Replication of Assay Ready Plates
(no buffer or biologicals)

Combine JIT tube picking with JIT plate replication

- **Avoid intermediate dilutions**
  - Small compound transfer amounts
- **Consolidate all compound transfer operations**
  - Complex equipment and expertise
  - Volatile solvent transfers
  - nL DMSO dispenses under inert environment
- **Guarantee quality, ready-to-use screening plates**

Conclusions

- **Choose automated storage systems with:**
  - Frozen, dry, inert environments
  - Fast tube picking in inert environment
  - JIT screening sets
- **Choose compound concentrations that don't precipitate**
- **Make screening plates at compound management facility**
  - Provide JIT individual-use, assay-ready plates
  - Eliminate storage of compounds at assay sites

Thanks for your attention!

vol % water	vol % DMSO	X_{water}	X_{DMSO}
0	100	0.000	1.000
5	95	0.172	0.828
10	90	0.305	0.695
20	80	0.497	0.503
30	70	0.628	0.372
34	66	0.670	0.330
50	50	0.798	0.202
80	20	0.940	0.060
90	10	0.973	0.027
95	5	0.987	0.013
98	2	0.995	0.005
99	1	0.997	0.003
100	0	1.000	0.000