BioGlobaX Inc.

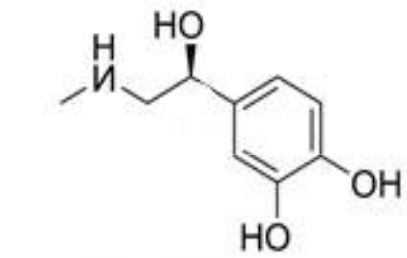
Analytical methodology and toolkit for improvement of quality attributes and process optimization of biopharmaceuticals

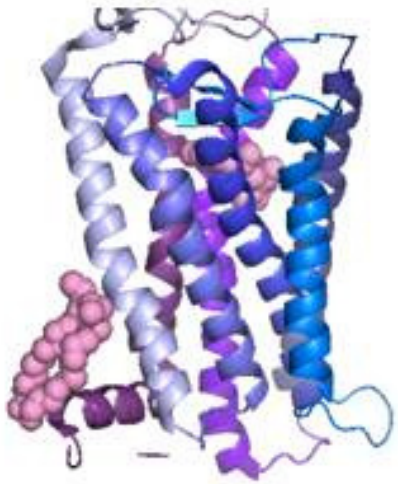
Anelia Atanassova, Sr. Scientist, BioGlobaX Inc., Toronto, Canada

August 10-12, 2015, London, UK

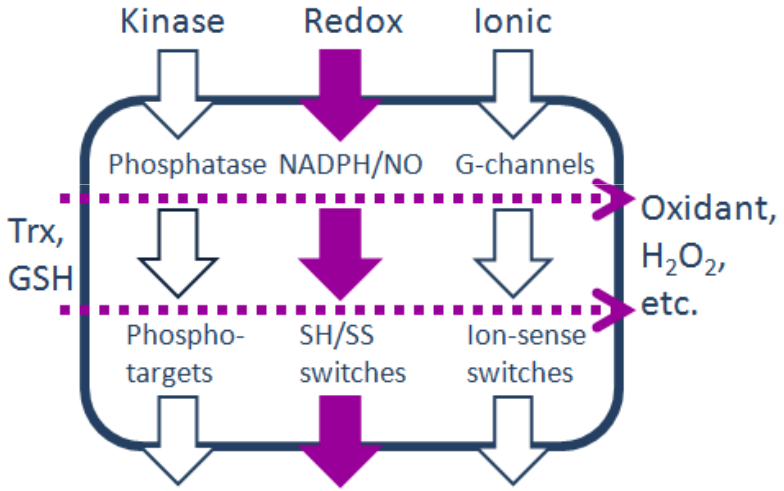
Drug Discovery

The Traditional:
Single Drug - Target





The "New" Biology:
X-omics

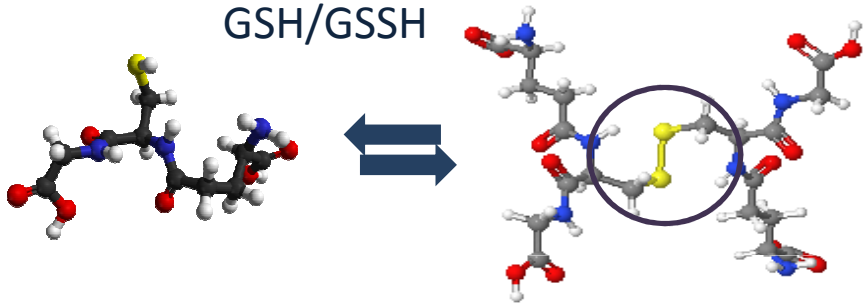


Adopted from J. of Internal Medicine 2010, 268 pp 432-448

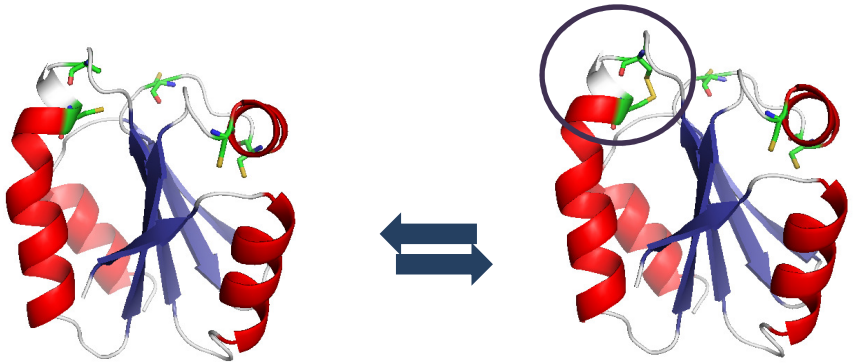
The Traditional: Thiol-based Redox Homeostasis

The "New" Biology: Cys Proteomics

GSH/GSSH



Thioredoxin

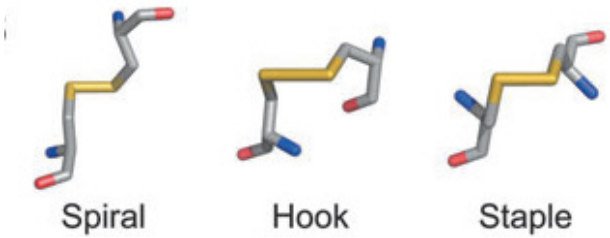


The Human Redox / Cys Proteome

214,000 Cys residues

5–12% - oxidized

H. proteins	3Ds	C-C bonds
Total	4,104	16,538



20 possible C-C configurations

Cook K & Hogg P (2013) Antioxid Redox Signal 18: 1987–2015

Reactive Oxygen Species

Metal Induced Oxidation and Degradation

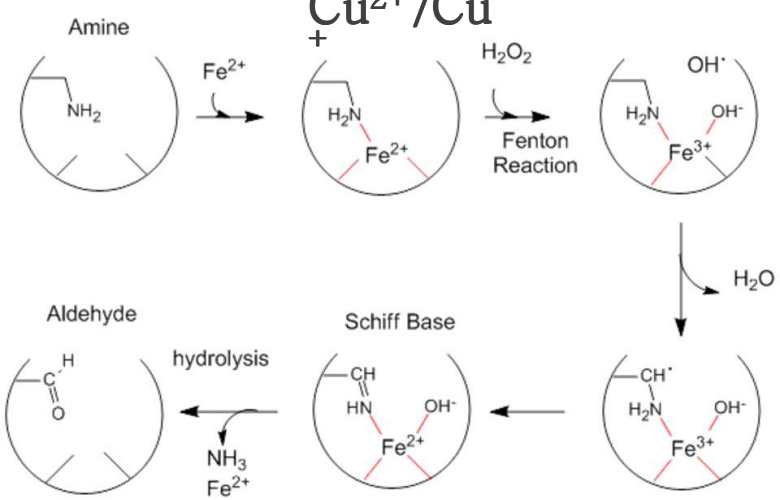
The Traditional:

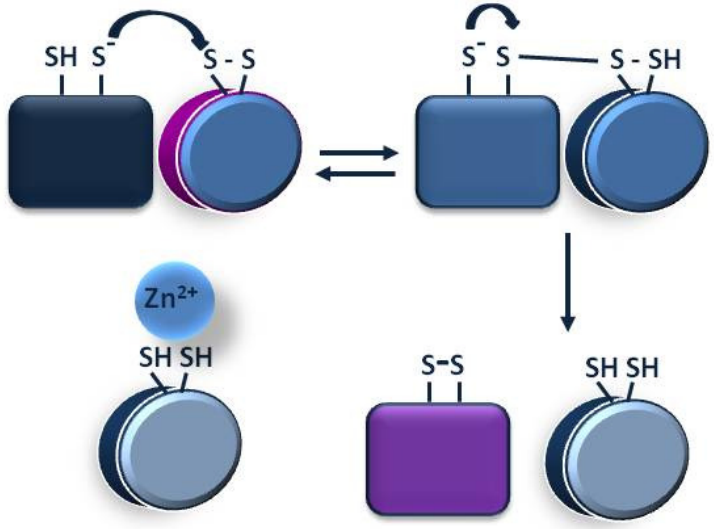
The "New" Biology:
Cys Proteomics

Fe^{3+}/Fe^{2}
+
Cu^{2+}/Cu
+

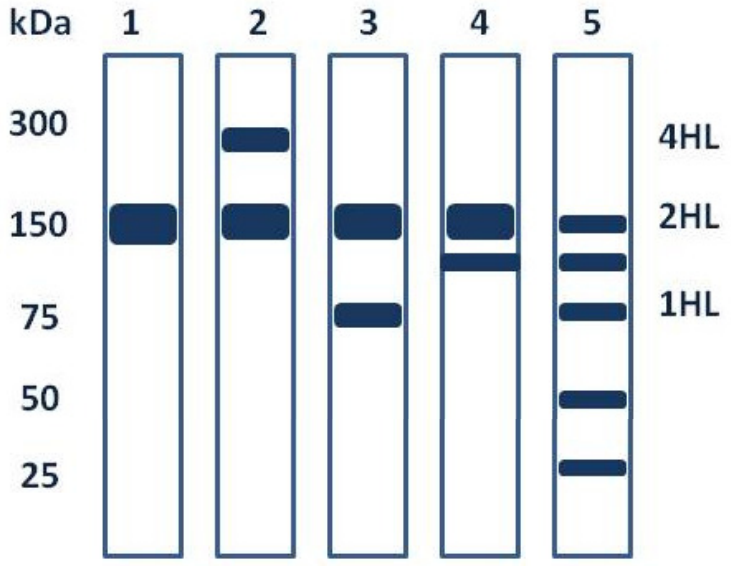
V/S

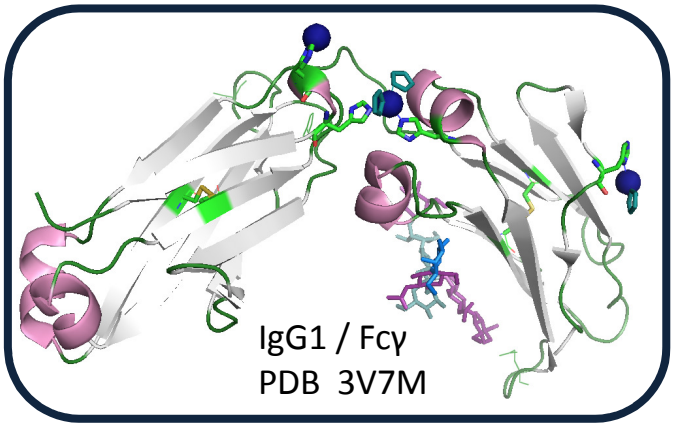
Zn^{2+}

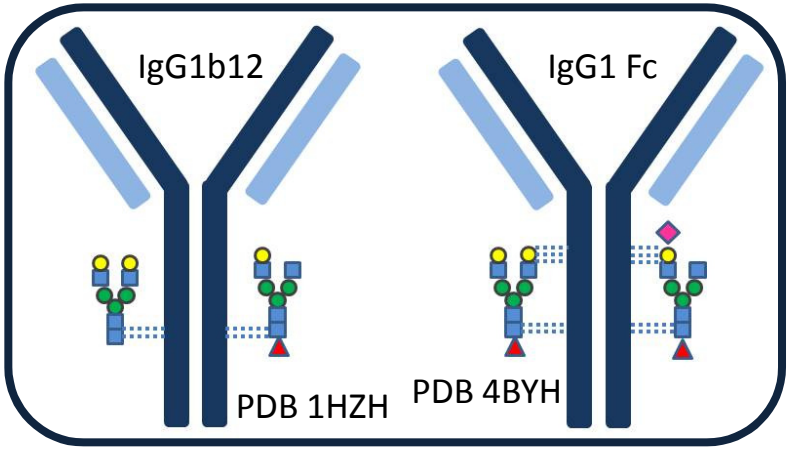


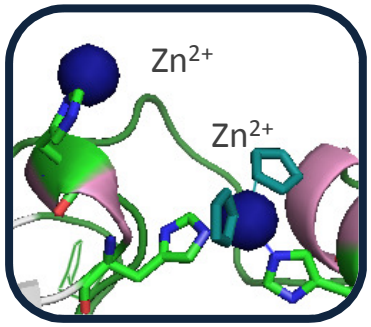


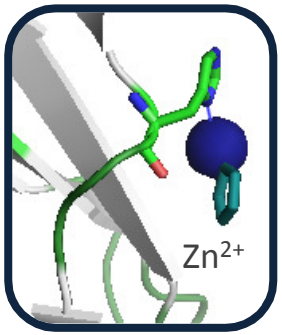
Antibodies: Real Data









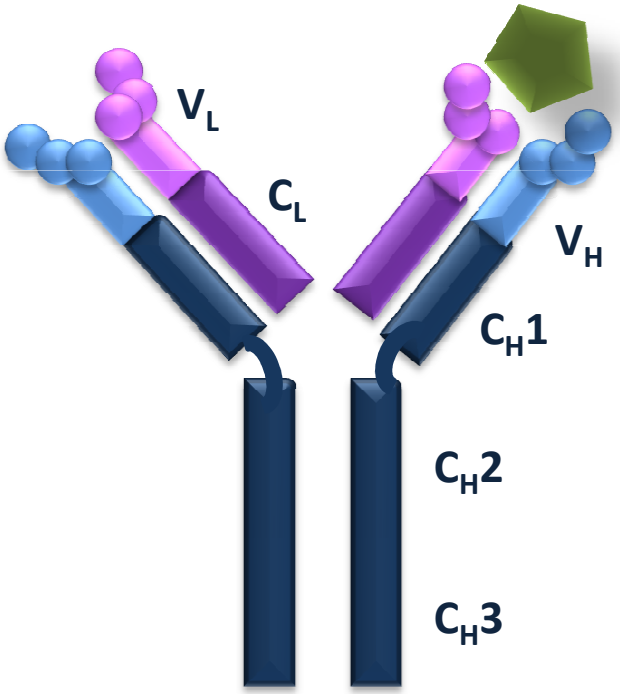


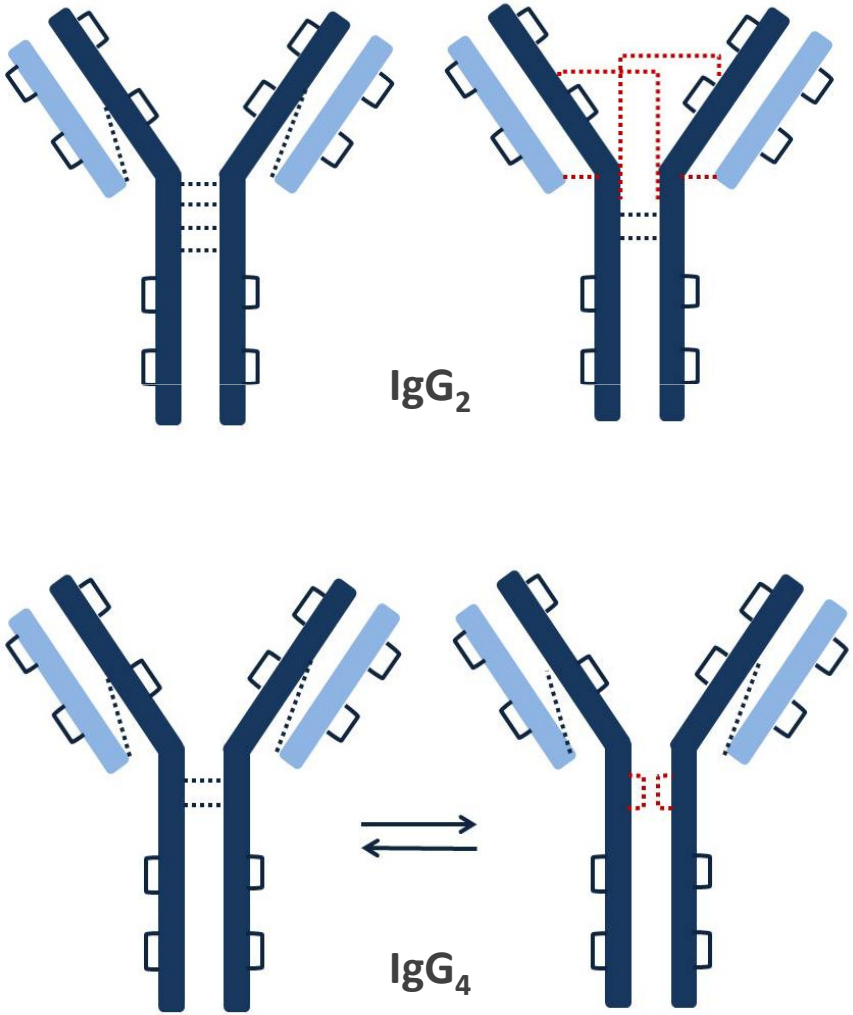
Sibéril S , Immunol.Lett. (2012) 143: 60-69

Antibodies

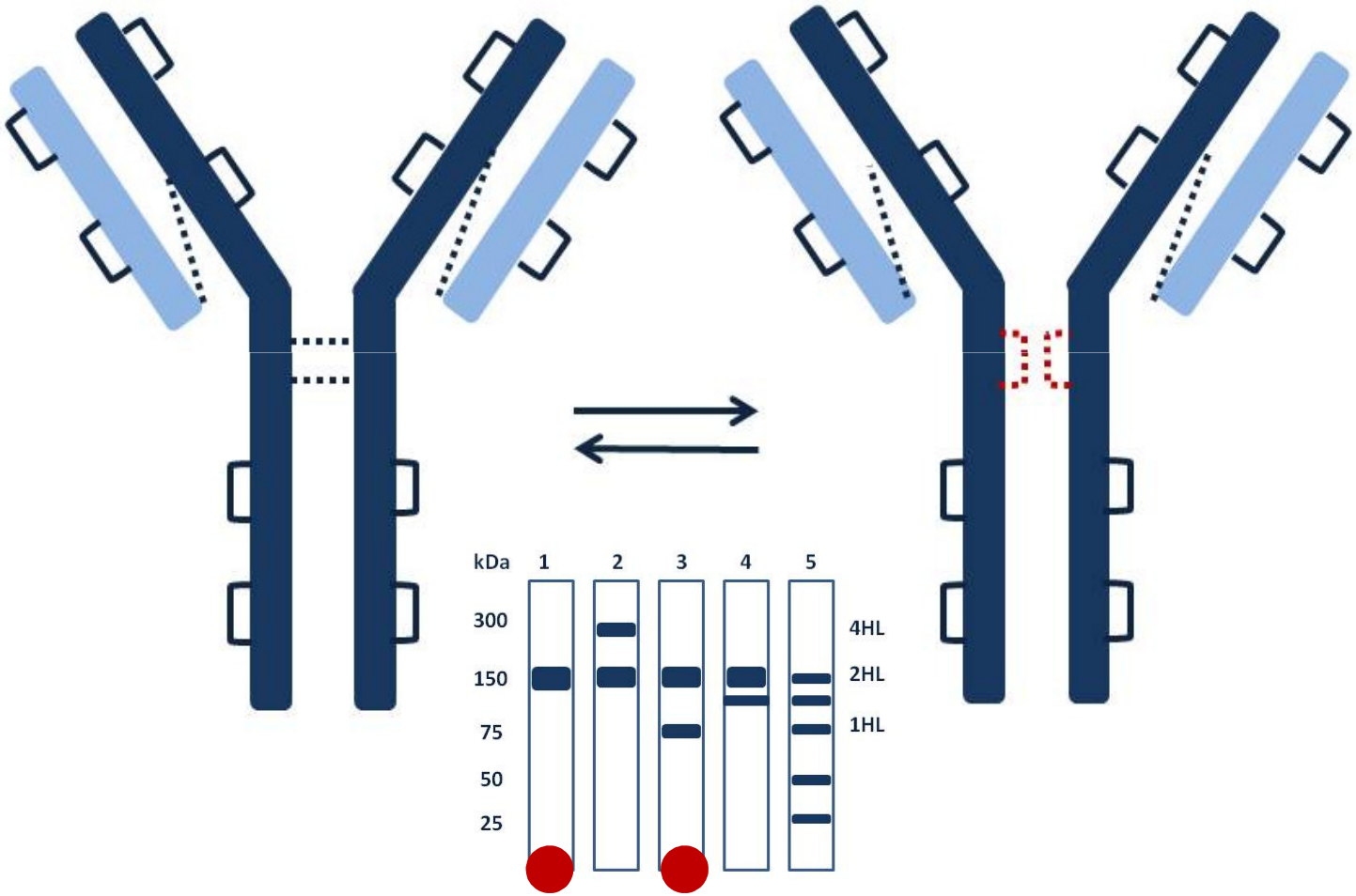
The "New" Biology:
Redox Proteomics

The Traditional Approach:





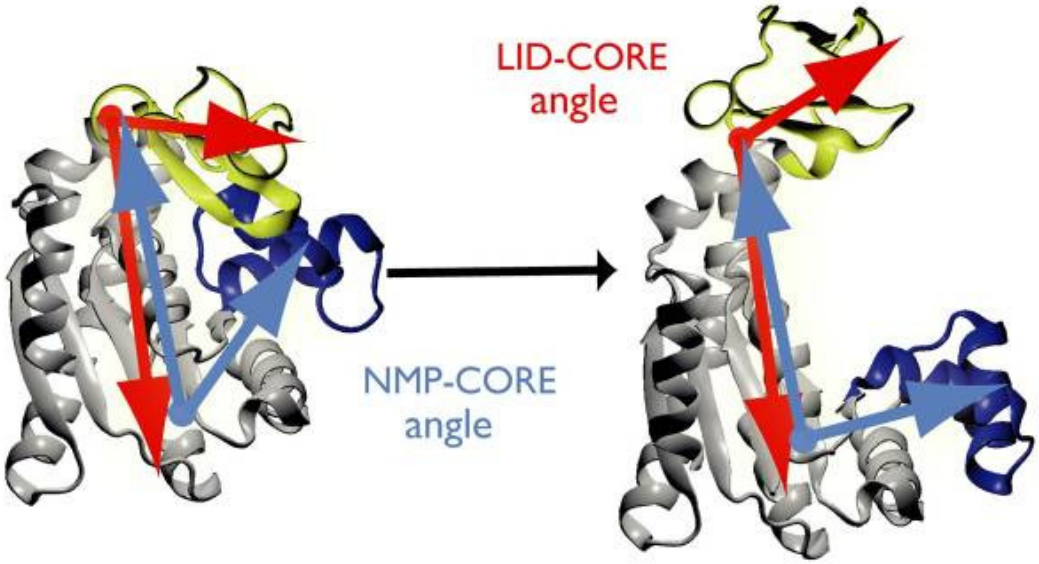
Antibodies Abnormalities

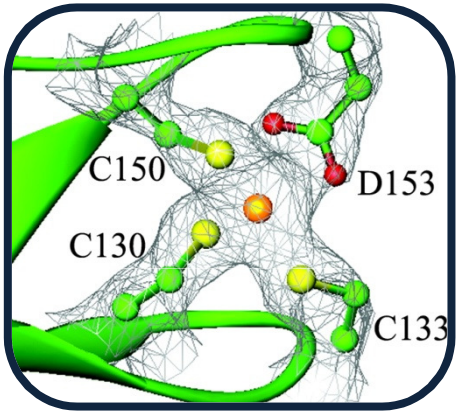


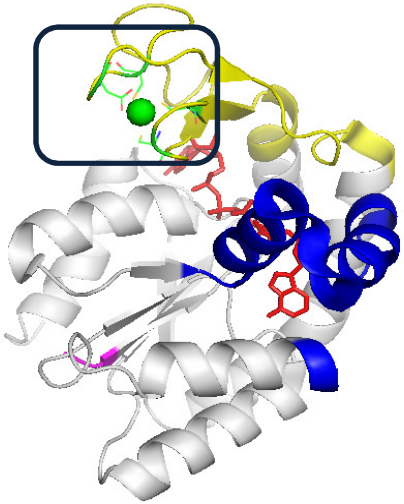
A Paradigm Shifts

Lessons from Enzymes: Adenylate Kinase

Mg^{2+} - ATP + AMP ↔ Mg^{2+} - ADP + ADP







The Power of Bioinformatics

```
                    10        20        30        40        50        60
                    |         |         |         |         |         |
ECOLI           --------MRIILLGAPGAGKGTQAQFIMEKYGIPQISTGDMLRAAVKSGSELGKQAKDI
Bacillus sub.   --------MNLVLMGLPGAGKGTQGERIVEDYGIPHISTGDMFRAAMKEETPLGLEAKSY
Mycobacterium t.--------MRVLLLGPPGAGKGTQAVKLAEKLGIPQISTGELFRRNIEEGTKLGVEAKRY
HUMAN1          MEEKLKKTKIIFVVGGPGSGKGTQCEKIVQKYGYTHLSTGDLLRSEVSSGSARGKKLSEI
HUMAN6          -----MLLPNILLTGTPGVGKTTLGKELASKSGLKYINVGDLAREEQLYDGYDEEYDCPI
                         :.: * ** ** *    : .. *   :..*:: *

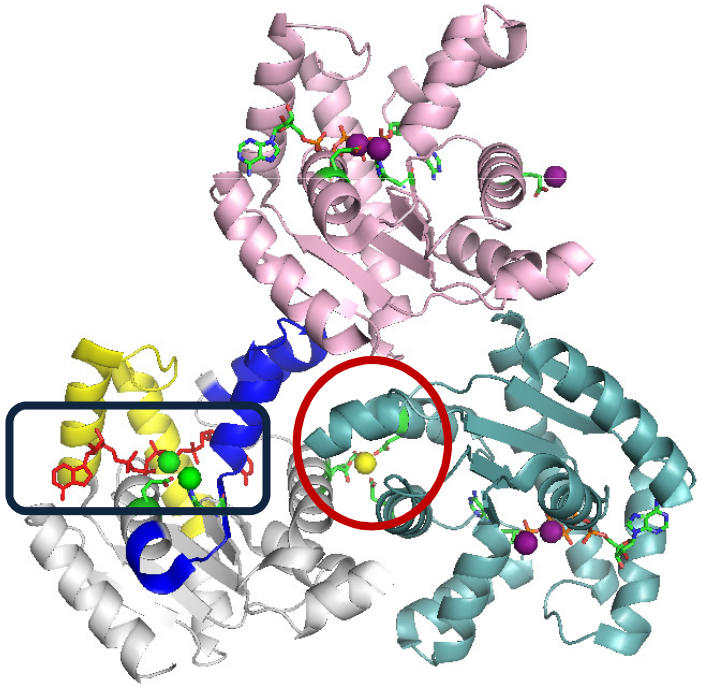
                    70        80        90        100       110       120
                    |         |         |         |         |         |
ECOLI           MDAGKLVTDELVIALVKER-IAQEDCRNGFLLDGFPRTIPQADAMKEA----GINVDYVL
Bacillus sub.   IDKGELVPDEVTIGIVKER-LGKDDCERGFLLDGFPRTVAQAEALEEILEEYGKPIDYVI
Mycobacterium t.LDAGDLVPSDLTNELVDDR-LNNPDAANGFILDGYPRSVEQAKALHEMLERRGTDIDAVL
HUMAN1          MEKGQLVPLETVLDMLRDAMVAKVNTSKGFLIDGYPREVQQGEEFERRIG----QPTLLL
HUMAN6          LDE-DRVVDEL------DN-QMREG---GVIVDYHG-----CDFFPER------WFHIVF
                ::  . *  :       :    . .   *.::* .       . : .          ::

                    130       140       150       160       170       180
                    |         |         |         |         |         |
ECOLI           EFDVPDELIVDRIVGRRVHAPSGRVYHVKFNPPKVEGKDDVTGEELTTRKDDQEETVRKR
Bacillus sub.   NIEVDKDVLMERLTGRRICSVCGTTYHLVFNPPKTPGICDKDGGELYQRADDNEETVSKR
Mycobacterium t.EFRVSEEVLLERLKGR-----------G-------------------RADDTDDVILNR
HUMAN1          YVDAGPETMTQRLLKRG--ETSG-------------------------RVDDNEETIKKR
HUMAN6          VLRTDTNVLYERLETR------G-------------------------YNEKKLTDNIQ
                 . .  : : :*:  *      *                          :: . .   :

                    190       200       210       220       230
                    |         |         |         |         |
ECOLI           LVEYHQMTAPLIGYYSKEAEAGNTKYAKVDGTKPVAEVRADLEKILG----
Bacillus  sub.  LEVNMKQTQPLLDFYS---EKG--YLANVNGQQDIQDVYADVKDLLGGLKK
Mycobacterium t.MKVYRDETAPLLEYYR-------DQLKTVDAVGTMDEVFARALRALGK---
HUMAN1          LETYYKATEPVIAFYE-----KRGIVRKVNAEGSVDSVFSQVCTHLDALK-
HUMAN6          CEIFQVLYEEATASYKEEIVHQLPSNKPEELENNVDQILKWIEQWIKDHNS
                              *             :    : .:       :
```

LID, ATP binding, AMP, NMP bind, **AMP**-R39; **ATP**-H79,R105, R109; *T17* binds Mg2+ in AK6human

Human AK1-trimer AP5 - Zn^{2+} PDB 1Z83



Analytical Toolkit



- **Methodology**
  - Bioinformatics
  - Targets and systems
  - Factors and instrument
- **Single, multiple and routine assays**
  - Sequence
  - Interactions

Analytical Toolkit





- **Methodology**
  - Bioinformatics
  - Targets and systems
  - Factors and instrument
- **Single, multiple and routine assays**
  - Sequence
  - Interactions

Factors to Consider:





Factors	Process	Product	
Cell type / strains	√	√	
Gene constructs	√	√	
pH, T^0, metabolites	√	√	
O_2, agitation	**√**	**√**	**Activity**
Media composition	**-**	**√**	**Stability**
Sequence and structure	**√**	**√**	**Yield**

Factors to Consider:





Factors	Process	Product
Buffers composition	-	√
Purification process	√	√
Additives	**-**	**√**
pH variations	**√**	**√**
O_2, H_2O_2, ROS	**√**	**√**
Impurities (metals)	**√**	**√**
Activity and Stability	**√**	**√**

Sequential VS DoE Approach

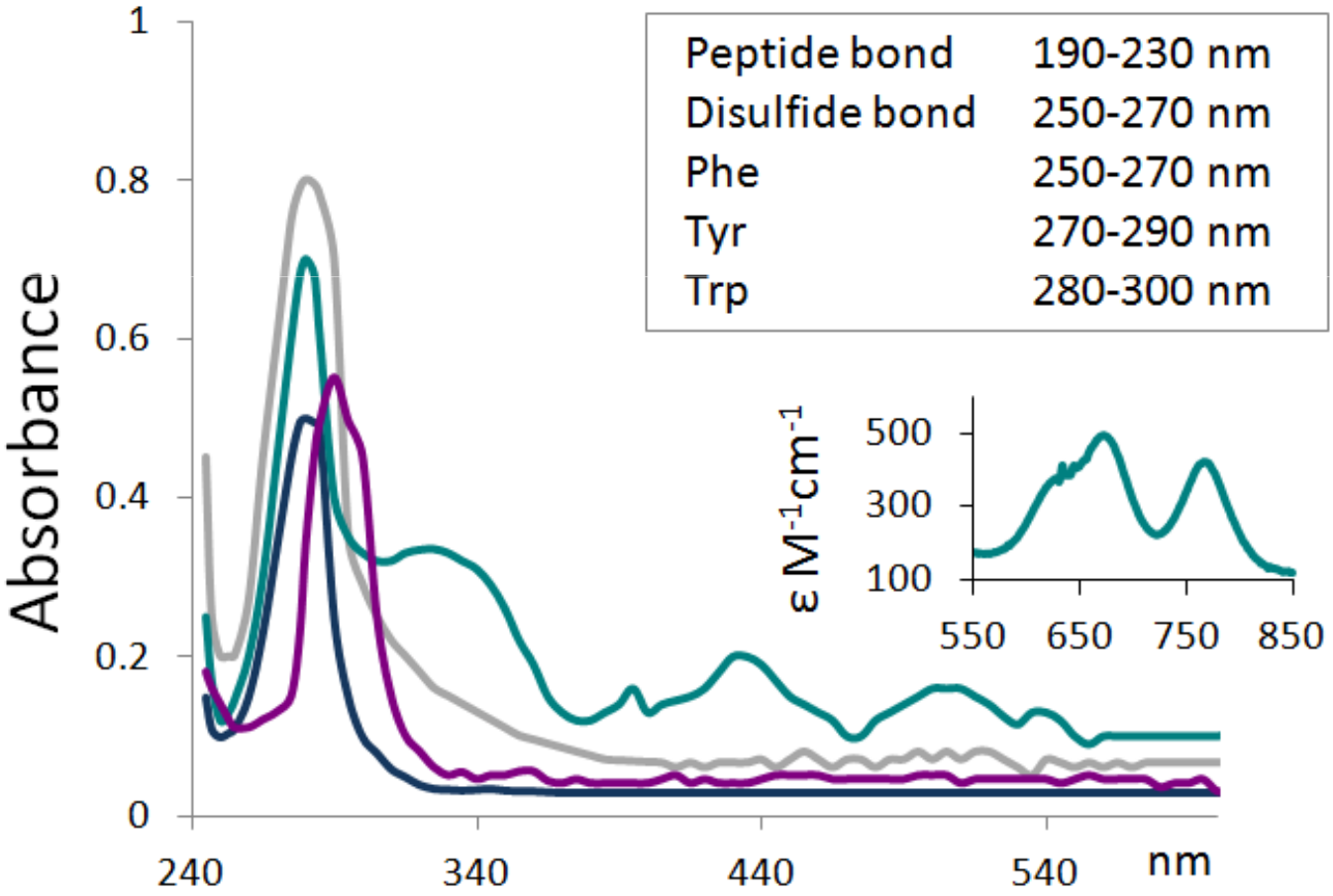


- Study ≥ two factors in parallel;
- Searching for correlations between them;
- More precise information;
- Fewer experiments.



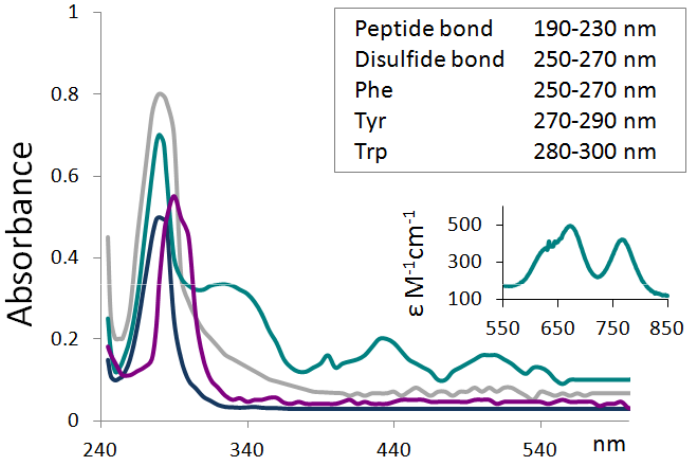
Cost Effective Assays

Absorption spectra of purified proteins



Cost Effective Assays

Absorption spectra of purified proteins

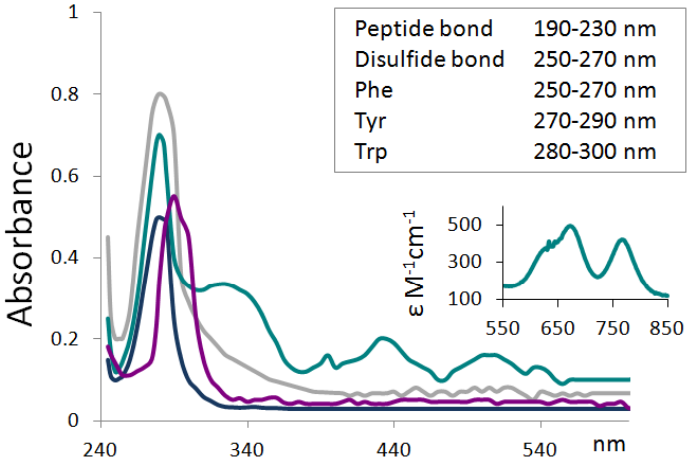


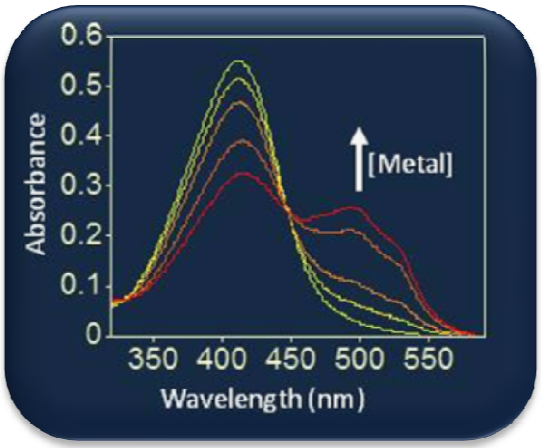
- ✓ Kinetics
- ✓ Molecular Weight

- ➢ Oligomers / Aggregates
- ➢ Conformational Changes
- ➢ Binding affinity / Specificity
- ➢ Small Molecule Coordination

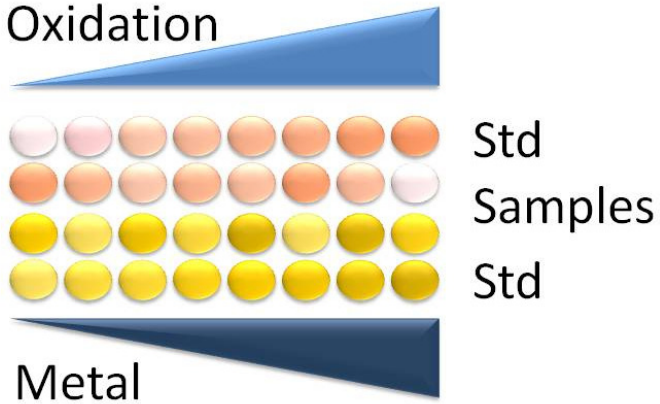
Cost Effective Assays

The Power of UV/Vis spectrometry



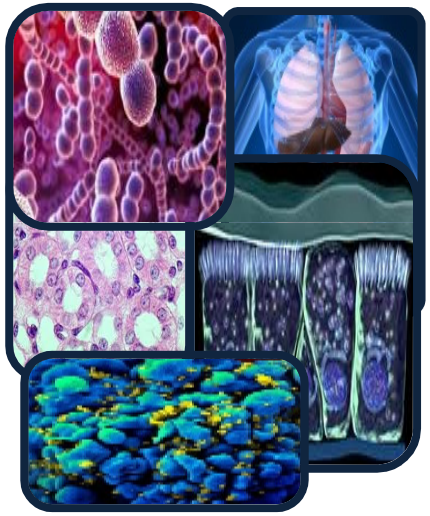


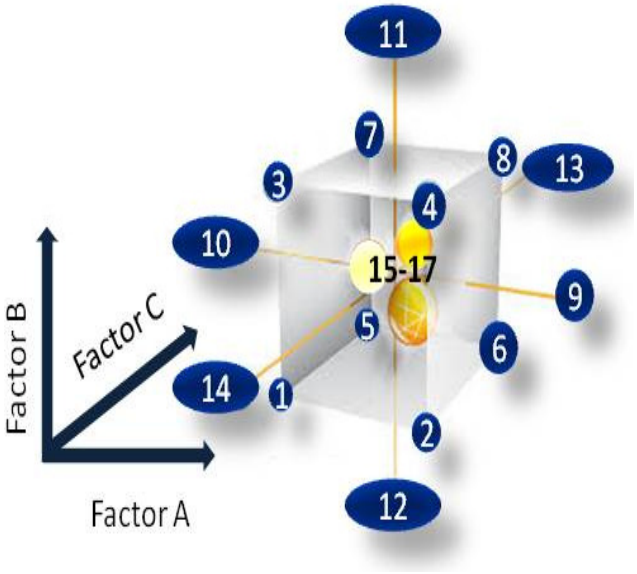
- ✓ Kinetics
- ✓ Molecular Weight
- ➢ **Oligomers / Aggregates**
- ➢ **Conformational Changes**
- ➢ **Binding affinity / Specificity**
- ➢ **Small Molecule Coordination**
- ➢ **Metal content**
- ➢ **Oxidation status**

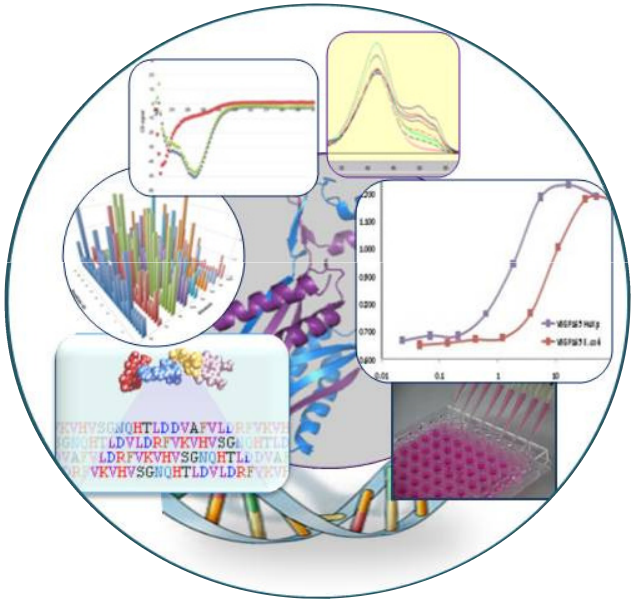


The Future

Relevant data collection / bioinformatics ↔ comparative analysis







Multidisciplinary DoE approaches

www.bioglobax.com

Acknowledgments

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