Computer-aided Protein Design

by combining automated reasoning and learning

Thomas Schiex

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JOBIM 2021

Why should we want to design proteins?

Eco-friendly chemical/structural nano-agents

- New drugs for health (human, animals, plants)
- New catalysts (environment, recycling, biofuels, food and feed, cosmetics…),
- New components for nanotechnologies
- Relying on inexpensive atomic level 3D-printers (bacterias, yeast, …)

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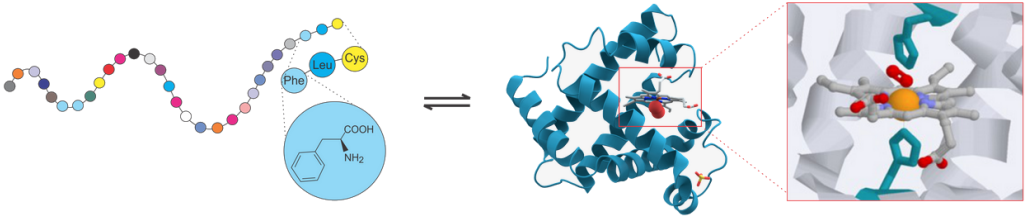

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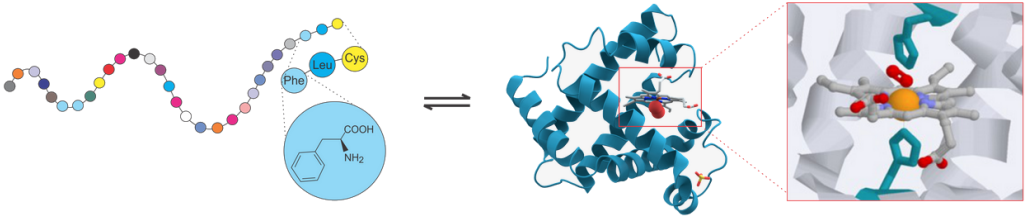

20^n sequences! Experimental techniques can only explore a very tiny fraction of it.

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The Computational Design Problem

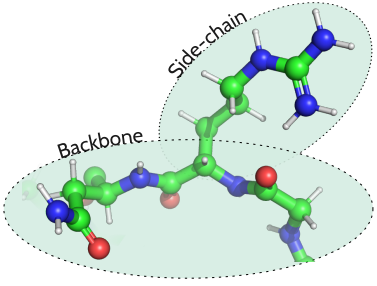
Informal definition (globular proteins)

Produce a sequence s of amino-acids that *spontaneously adopts* a conformation X that *performs some function*.

What defines a conformation ?

Conformation

- backbone: dihedral angles ϕ_i, ψ_i
- sequence: amino-acid choice s_i
- side-chains: torsion angles χ_{ij}



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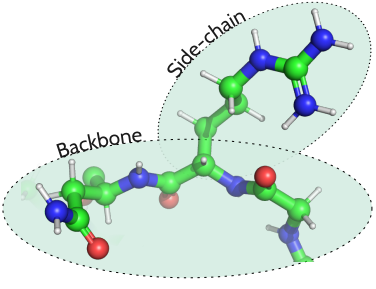

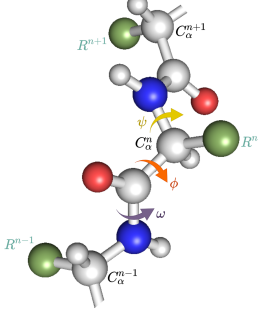

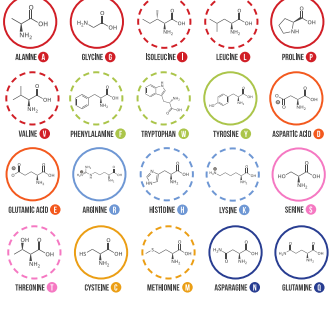

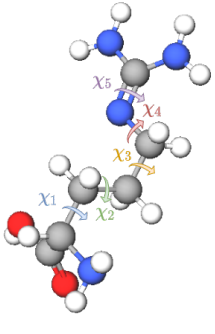

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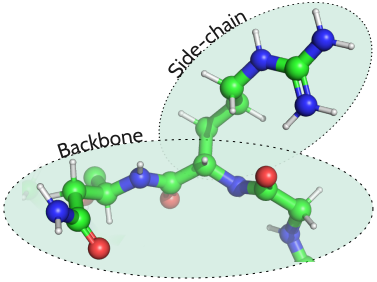

Challenging space to explore

- very high dimensionality, continuous variables ($\phi_i, \psi_i, \chi_{ij}$)
- discrete set of possible sequences s (size 20^n)

Atomic forces and entropic effects

- Chemical bonds geometries
- Inter atomic forces (electrostatics, polar, van der Waals…)
- Solvent effects

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Thermodynamics[2]

- The stability of a sequence s in a given conformation X can be estimated through a real valued energy function $E(\mathrm{s}, X)$.

$$p_{\mathrm{s}}(X) \propto e^{-\frac{E(\mathrm{s},X)}{k_B T}}$$

- intractable non convex $E(\mathrm{s}, X)$ (free energy, quantum mechanics)
- Plus **extra requirements** for the function itself (sequence, geometry, flexibility…).

The "rigid backbone, discrete rotamers, pairwise decomposable energy" problem

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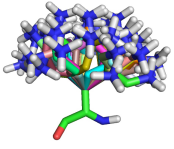
Rotamer libraries: Tuffery,[28] Penultimate,[15] Dunbrack[25] …

Catalog of (amino acid, side-chain conformations) pairs build from the PDB (typically 400 or more rotamers)



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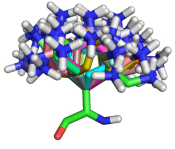

We need to minimize (+ fitness) Precomputed tables

$$E(\mathbf{s}, X) = E_{\varnothing} + \sum_{i=1}^{n} E_i(i_r) + \sum_{(i,j)\in I} E_{ij}(i_r, j_s)$$

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Forgetting all approximations

Even if $(\mathbf{s}, \chi)$ minimizes E on (ϕ, ψ), a better backbone configuration for $\mathbf{s}$ may exist.

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Extra checks

1. Post-hoc continuous minimization of ϕ, ψ, χ (nicely dealt with by OSPREY[7,10])
2. Molecular dynamics simulations (expensive).
3. Forward folding: predict the structure from $\mathbf{s}$.

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It works[22]

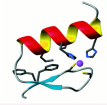
- There are less than 2,000 known folds for many more sequences.
- Secondary structure elements and hydrophobic packing constrain the space.
- We are in control and can make designs very predictable (forward folding).

1985

Calmodulin-binding peptide

[DeGrado et al. 1985]

1997



Zinc Finger

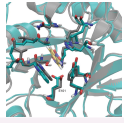
[Dehiyat & Mayo 1997]

2003

Novel Topology (top7)

[Kuhlman et al. 2003]

2008



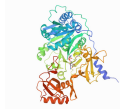
Functional Enzyme

[Rothlisberger et al. 2008]

Enzyme for Multi-Step Reaction

[Jiang et al. 2008]

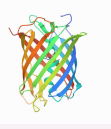
2009



Enzyme activity

[Chen et al. 2009]

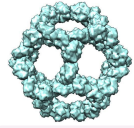
2011



Longer Emission Wave Length Fluorescence

[Chica et al. 2011]

2016



Self-Assembling Nanocage

[Hsia et al. 2016]

2019

Auto-Assembling Symmetrical Protein

[Niguchi et al. 2019]

NP-hard[19] (intractable?) — **Precomputed tables**

$$E(\mathbf{s}, X) = E_{\varnothing} + \sum_{i=1}^{n} E_i(i_r) + \sum_{(i,j)\in I} E_{ij}(i_r, j_s)$$

- mostly solved by Monte-Carlo algorithms (Rosetta)[14]
- NP-hard: standard excuse for approximate modeling or (meta)-heuristics.

Minimizing E

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Using Cost Function Network (CFN) algorithms — **github.com/toulbar2**

- Intense progress in AI on logical/Boolean reasoning (200TB theorem proof[13])
- CFN use automated reasoning algorithms extended to numerical functions.[11]
- Can still handle logical information (constraints)

Cost Function Network (a type of Graphical model)

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- a sequence X of discrete variables x_i, domain D_i

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Graphical models?

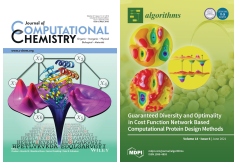
- The interactions captured by the model can be represented as a graph
- Variables are vertices
- They are connected by an edge if they interact (participate together in a function)
- Cost Function Networks are closely related to Markov Random Fields

Exact vs. Stochastic search (See JFPC'21 slides)

Large input (> 1GB) **NP-hard problem**

Toulbar2 is able to…

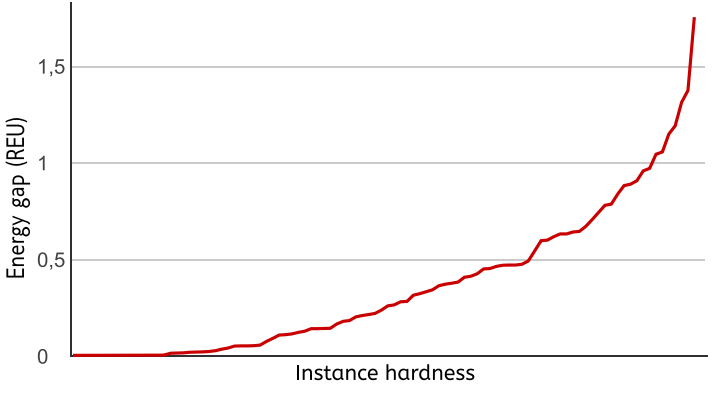
- provide a proven zero/bounded gap minimum energy solution[27]
- exhaustively enumerate sequences close to it
- provide sequence libraries with guaranteed diversity.[20]
- in sequence-conformation spaces of size $> 10^{400}$



Rosetta's Monte Carlo Simulated Annealer increasingly fails to find the optimal sequence[a]

[a] David Simoncini et al. "Guaranteed Discrete Energy Optimization on Large Protein Design Problems". In: *Journal of Chemical Theory and Computation* 11.12 (2015), pp. 5980–5989. DOI: 10.1021/acs.jctc.5b00594.

Unbounded error



Asymptote: **Size matters!**

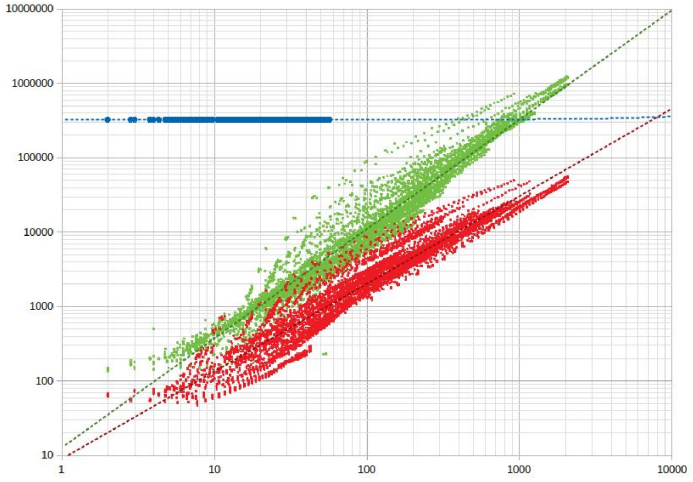
Asymptotic convergence can be arbitrarily slow…

Guaranteed Discrete Energy Optimization on Large Protein Design Problems

David Simoncini[†], David Allouche[†], Simon de Givry[†], Céline Delmas[†], Sophie Barbe[‡§⊥], and Thomas Schiex[*†]

JCTC Journal of Chemical Theory and Computation

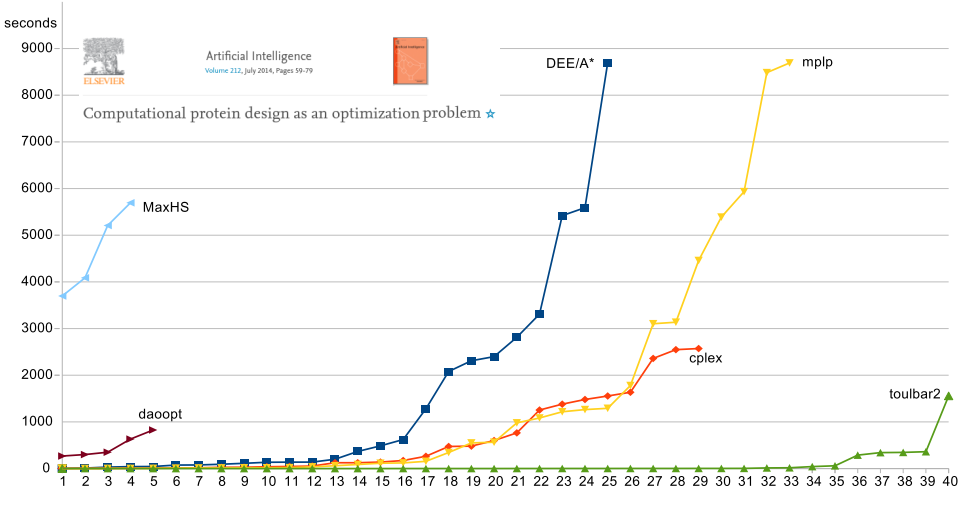
Quantum computing (DWave),Toulbar2 & SA[1]



DWave approximations		*kcal/mol*
gap > 1.16, 90% of the time	> 4.35, 50% of the time	> 8.45, 10% of the time

[1] Vikram Khipple Mulligan et al. "Designing Peptides on a Quantum Computer". In: *bioRxiv* (2019), p. 752485.

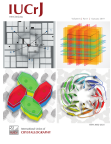
Toulbar2 vs. CPLEX, MaxHS…(real instances)



# of instances solved (*X*) within a per instance cpu-time limit (*Y*)

Designing a self-assembling β-propeller

Coll. A. Voet (KU Leuven), D. Simoncini[17]



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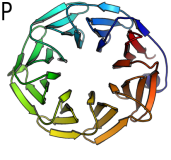

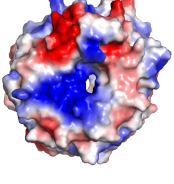

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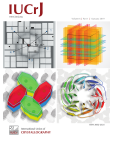

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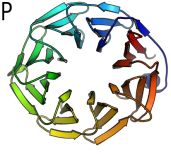

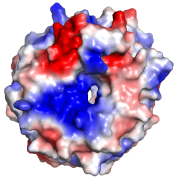

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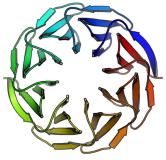

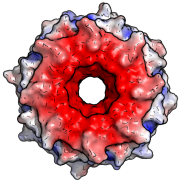

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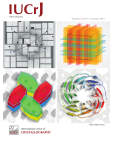

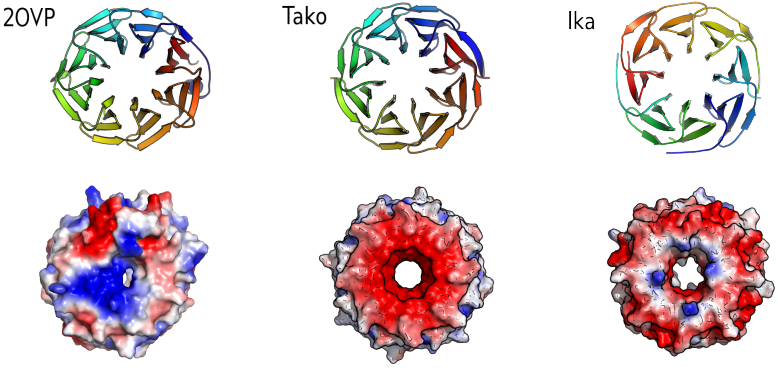

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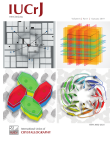

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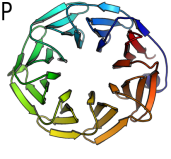

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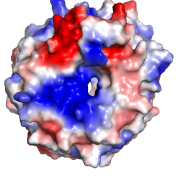

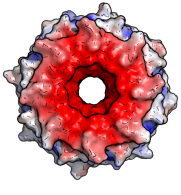

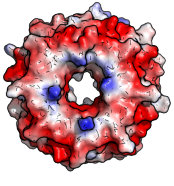

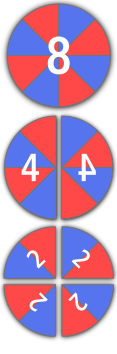

Using this new computational capacity for better modeling[2]

Capturing protein flexibility[4] through Multi-state design

Find a sequence that stabilizes multiple structures at the same time

What for?

- Bound and unbound conformations for enzymes, or binders
- Conformational switches
- All proteins are flexible!
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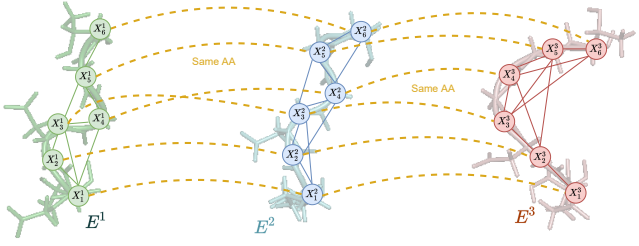

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Improves design quality at reasonable computational costs[3]

How correctly does it reconstruct natural proteins?

- Native sequence recovery (NSR)

Improvement over traditional Single State Design

	NMR	X-ray
NSR	+ 15,6 %	+8 %

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Injecting Machine Learned information

Energy is imperfect

- Approximations: solvent effect…
- Ignored: polarisability, expressability…
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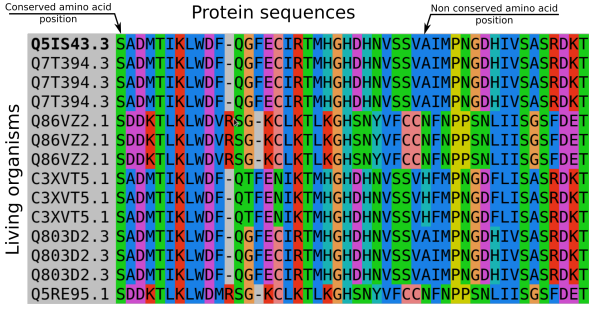
Evolutionary information

- Use a Multiple alignment of similar proteins (homologs)

A multiple alignment with conserved positions



- Used to force amino acid choice (constraint) at conserved positions.
- or bias by "Position Specific Score Matrices" (frequency / position)

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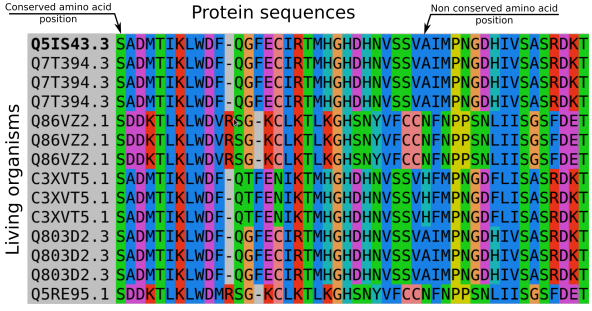


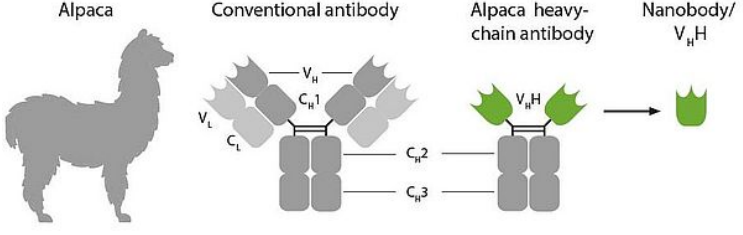
- Used to force amino acid choice (constraint) at conserved positions.
- or bias by "Position Specific Score Matrices" (frequency / position)

Algorithms for contact-map predictions[24] (MRF estimation)

- Identifies how close residues prefer to co-vary
- Combine this information with energy (linear combination)

Designing a new nanobody scaffold (coll. TBI, INSERM-CRCT)

- Using Rosetta score function and rotamer library
- Trying to satisfy several constraints (originality, composition…)
- Multi-state design:[29] multi-CDR loops compatible
- MSA-extracted evolutionary preferences



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Limited experimental power

- Over 6 sequences designed without evolutionary information: 3 expressed
- Over 3 sequences designed with evolutionary information: 3 expressed
- Much more power in recent Science/PLOS papers[21,23]
- Positive results on three environmental-friendly enzymes (coll. S. Barbe, TBI).

CFNs bring ML and Automated reasoning together

With Cost Function Network algorithms, one can…

- Model the problem as a CFN: knowledge (logic, energy)

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- Combine the models by scaling/adding/connecting them together
- Add further design constraints/preferences: desired properties
- Solve them with toulbar2 to get your new design (NP-hard)

Open source

https://github.com/toulbar2/toulbar2
https://github.com/toulbar2/CFN-learn

Exciting DL revolution in Protein Design too

Rigid body DL design approaches[8]

- Strongly inspired from NLP approaches (sequence, translation: transformers,...)
- Enriched by 3D geometry: SE(3) equivariance
- Coarse grained approches (backbone atoms only) mapping a backbone to a sequence
- Learning $P(s_i = AA|\mathrm{environment})$ for design[1,12]

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Design requires to impose 'fitness' constraints on the output

- Non trivial for Deep Learning
- Variational auto-encoders latent space interpolation[6]
- Driven generative adversarial networks[9]

Unleashing the power of DL-based structure prediction for design

Inverting DL Structure predictors (TrRosetta/RosettaFold[3]) α-Fold 2

- These networks somehow capture the sequence/structure relationship
- Back-propagation from a (sequence(s), structure) pair: symbolic sequence gradients
- Seems to be able to fight the "ill-posed problem" issue[18]
- Best performance obtained by injecting DL prediction as energy bias terms
- These terms can also be swallowed by Cost Function Networks.

Conclusion

- Designing new proteins with new functions can have strong real-world impact
- Design requires to assemble knowledge, experience (data), and constraints on the output
- Cost Function networks algorithms offer new capacities for CPD (NP-hard$\neq$ intractable)
- They rigourously combine physical energy with design constraints
- And can also swallow Machine/Deep Learned information
- Deep Learning may contribute to solve the long standing issue of 'alternative structures"
- But still needs to improve its capacities to satisfy output constraints

We still need to get rid of plenty of assumptions: come and dive in the amazing world of molecular design!

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Protein Design

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J. Cortes (LAAS/CNRS),…

My apologies to those missing in these lists. Even imperfect lists seem better than no list

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