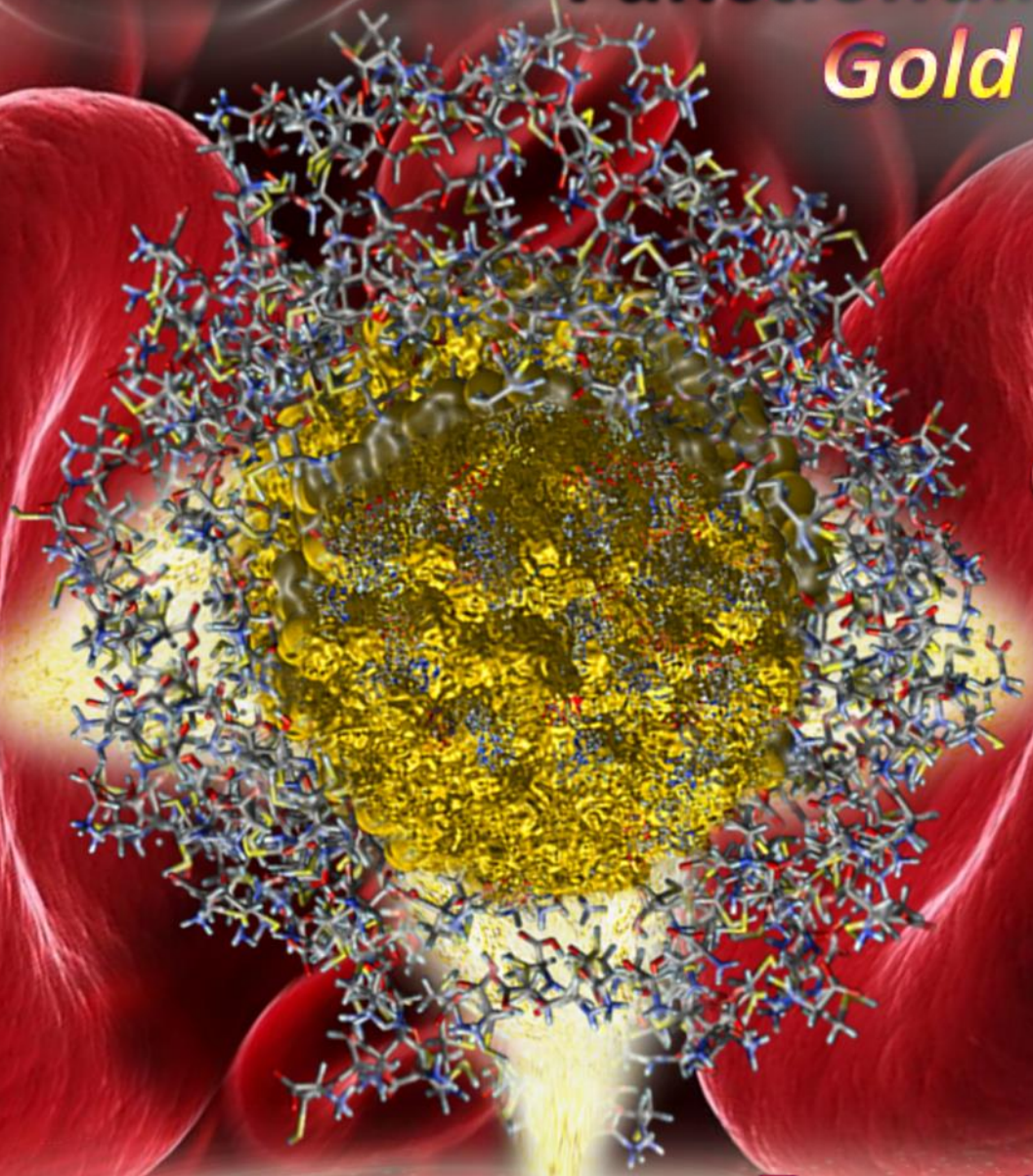

Looking into the dark side ...

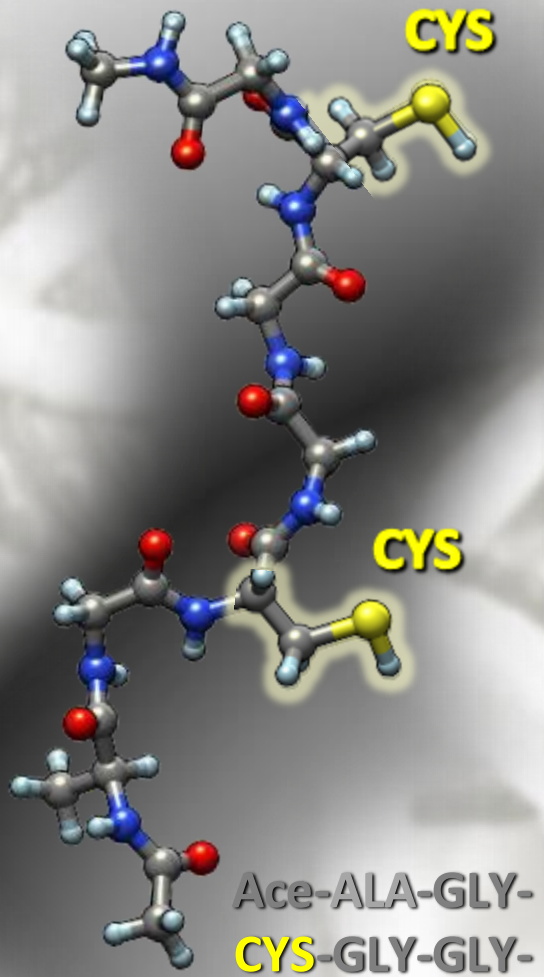
Susanna Monti

Functionalization of Gold Supports with Cysteine-Based Peptides

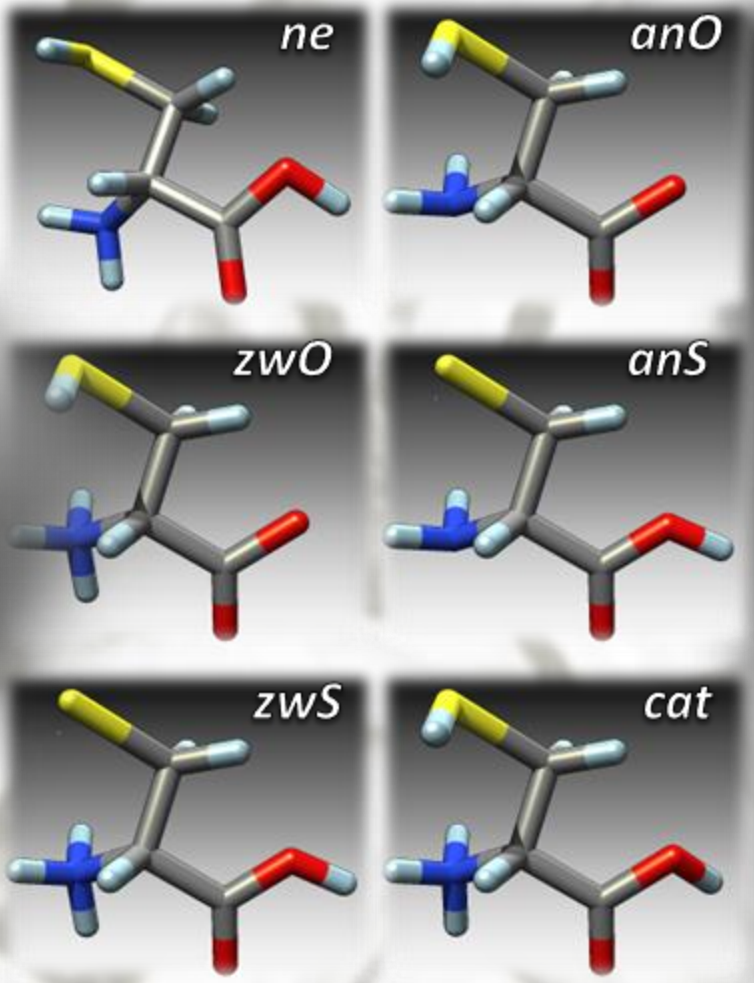


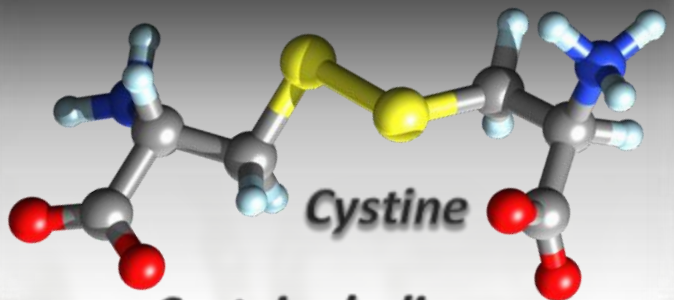
ReaxFF MDs

Cysteine & Cysteine-Based Peptides

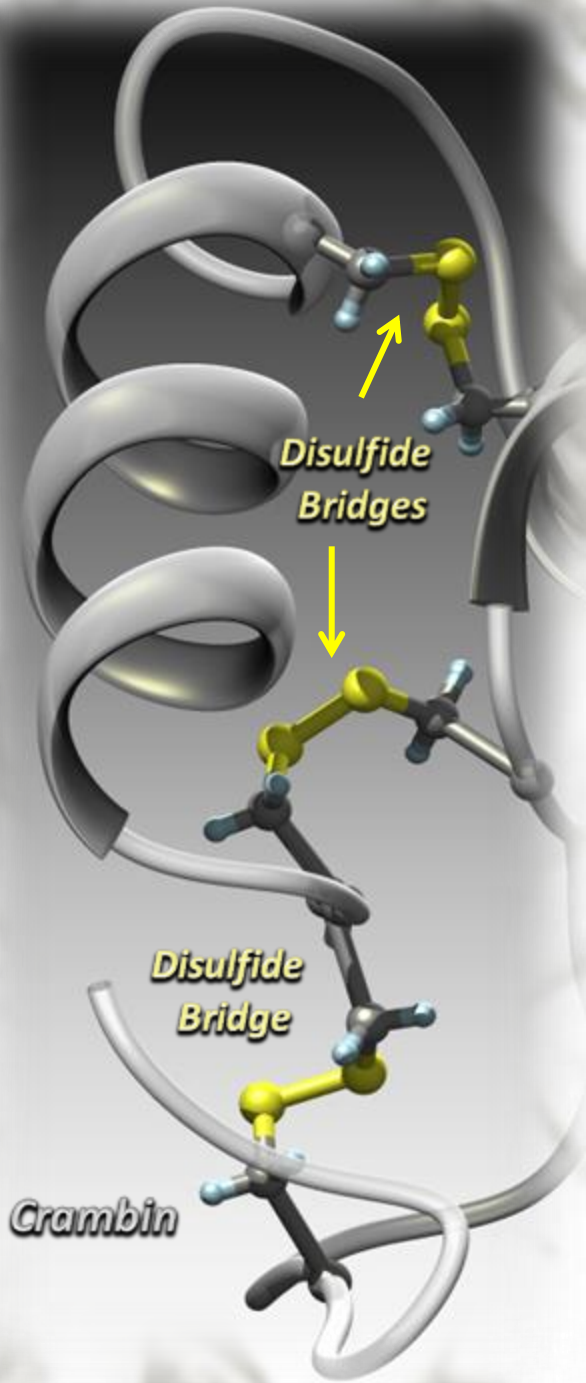


Ace-ALA-GLY-
CYS-GLY-GLY-
CYS-GLY-Nme





Cysteine's dimer

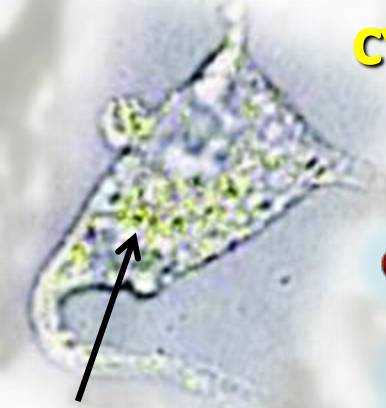


Crambin

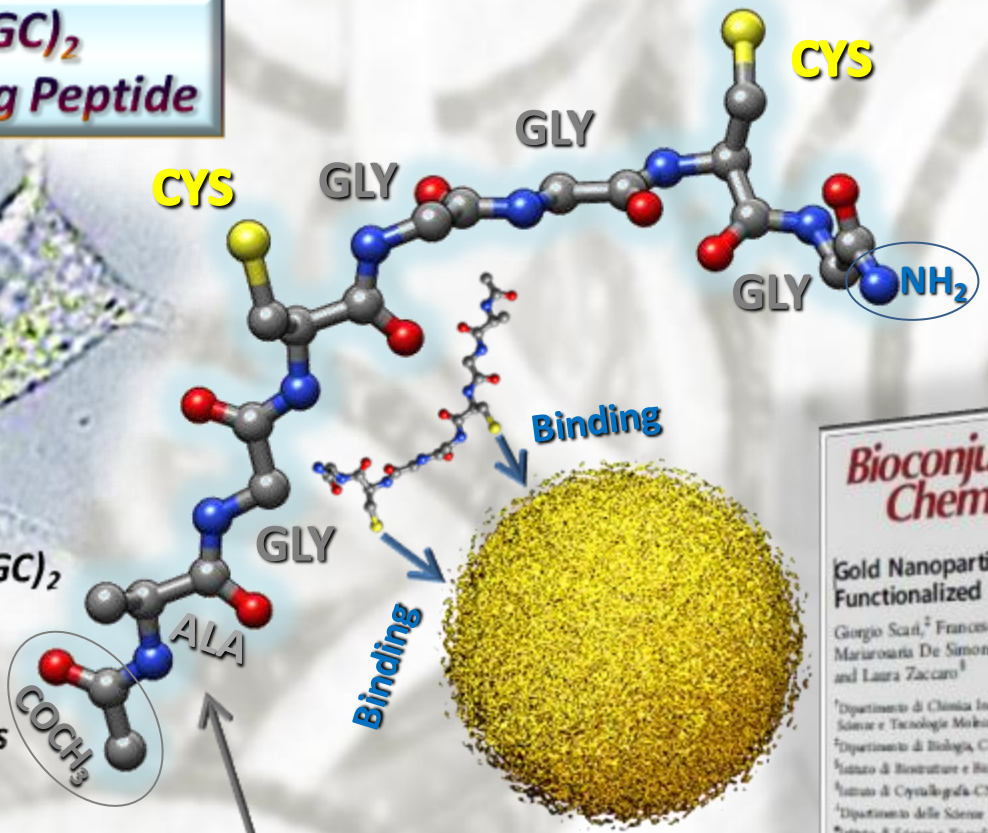
Ace-ALA-GLY-CYS-GLY-GLY-CYS-GLY-NH_2

Experimental Evidence

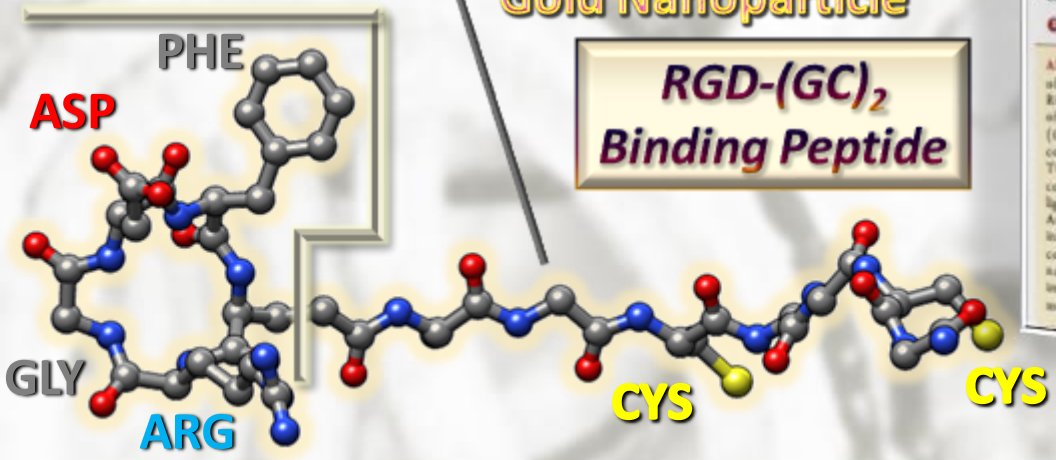
$(GC)_2$ Binding Peptide



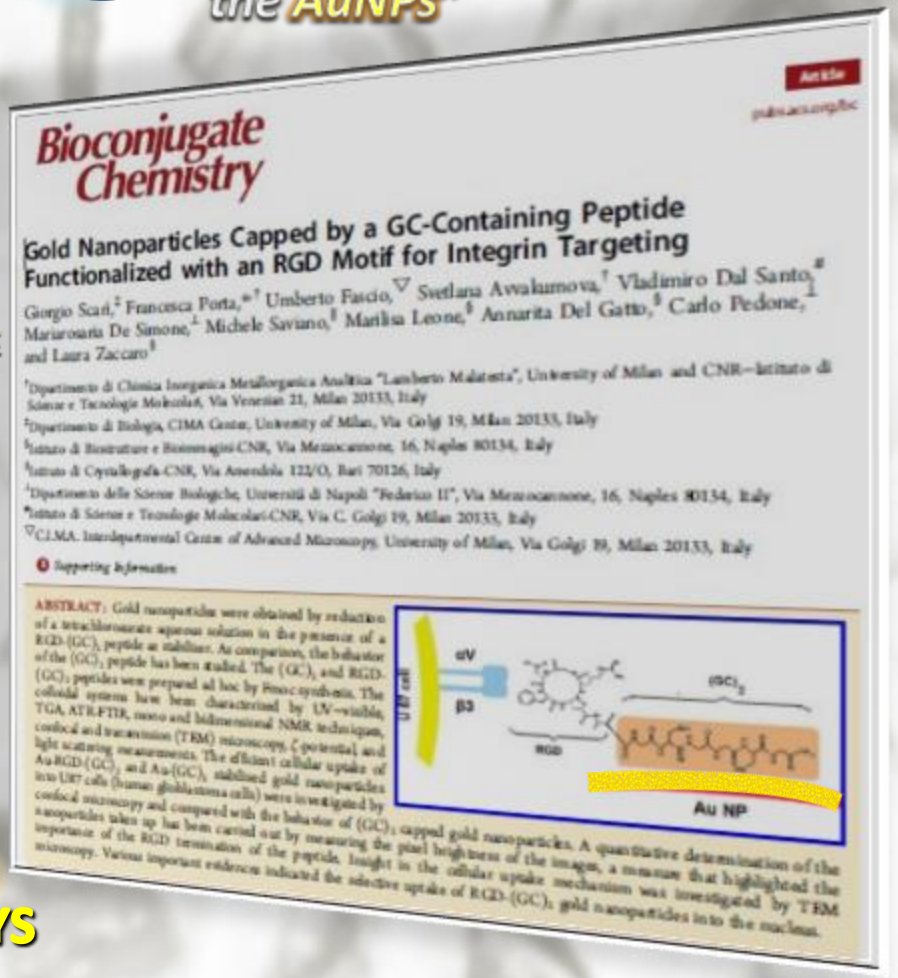
Au-RGD-$(GC)_2$ NPs taken by cancer cells



$RGD-(GC)_2$ Binding Peptide



"NMR data clearly indicate that the AGCGGCG tail of RGD functionalized peptide is responsible for binding to the AuNPs"



Bioconjugate Chemistry

Gold Nanoparticles Capped by a GC-Containing Peptide Functionalized with an RGD Motif for Integrin Targeting

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[‡]Dipartimento di Biologia, CIMA Centre, University of Milan, Via Golgi 19, Milan 20133, Italy
[§]Istituto di Biostrutture e Bioimmagini-CNR, Via Mezzocannone, 16, Naples 80134, Italy
[⊥]Istituto di Cristallografia-CNR, Via Amendola 122/O, Bari 70126, Italy
[‡]Dipartimento delle Scienze Biologiche, Università di Napoli "Federico II", Via Mezzocannone, 16, Naples 80134, Italy
[#]Istituto di Scienze e Tecnologie Molecolari-CNR, Via C. Golgi 19, Milan 20133, Italy
[▽]C.I.M.A. Interdepartmental Centre of Advanced Microscopy, University of Milan, Via Golgi 19, Milan 20133, Italy

Supporting Information

ABSTRACT: Gold nanoparticles were obtained by reduction of a tetrachloroaurate aqueous solution in the presence of a RGD-$(GC)_2$ peptide as stabilizer. As comparison, the behavior of the $(GC)_2$ peptide has been studied. The $(GC)_2$ and RGD-$(GC)_2$ peptides were prepared ad hoc by Fmoc synthesis. The colloidal systems have been characterized by UV−visible, TGA, ATR-FTIR, mono and bidimensional NMR techniques, confocal and transmission (TEM) microscopy, ζ-potential, and light scattering measurements. The efficient cellular uptake of Au-RGD-$(GC)_2$ and Au-$(GC)_2$ stabilized gold nanoparticles into U87 cells (human glioblastoma cells) were investigated by confocal microscopy and compared with the behavior of $(GC)_2$ capped gold nanoparticles. A quantitative determination of the nanoparticles taken up has been carried out by measuring the pixel brightness of the images, a measure that highlighted the importance of the RGD termination of the peptide. Insight in the cellular uptake mechanism was investigated by TEM microscopy. Various important evidences indicated the selective uptake of RGD-$(GC)_2$ gold nanoparticles into the nucleus.


D4RK
ReaxFF

ReaxFF Parametrization and Validation

Tentative parameters developed to reproduce QC data starting from the old force fields

Required descriptors: Au/S/O/N/C/H

In ADF: Au/S/O/C/H

AuCSOH.ff: (Au/C/S/O/H)
J.A. Keith, D. Fantauzzi, T. Jacob, and A.C.T. van Duin
Reactive forcefield for simulating gold surfaces and nanoparticles
Physical Review B (2010) 81, 235404-1/235404-8

AuSCH_2011.ff: (Au/S/C/H) and an improvement (2013)
T.T. Jarvi, A.C.T. van Duin, K. Nordlund, and W.A. Goddard III,
Development of Interatomic ReaxFF Potentials for Au–S–C–H Systems
J. Phys. Chem. A, 115, 10315–10322 (2011)

The original Au-Au parameters were extended by three publications:

Au/O: K. Joshi, A.C.T. van Duin, and T. Jacob
Development of a ReaxFF description of gold oxides and initial application to cold welding of partially oxidized gold surfaces
Journal of Materials Chemistry 20, (2010), 10431-10437

Au/C/S/H: T.T. Jarvi, A.C.T. van Duin, K. Nordlund, and W.A. Goddard
Development of interatomic ReaxFF potentials for Au-S-C-H systems
Journal of Physical Chemistry C 115, (2011), 10315-10322

C/O/H/S: Rahaman, O., van Duin, A. C. T., Goddard, W. A., III, and Doren, D. J.
Development of a ReaxFF reactive force field for glycine and application to solvent effect and tautomerization
Journal of Physical Chemistry B 115 (2011), 249-261

The forcefield does not include Au/N parameters

The old training sets have not been considered to develop the modified parameters used to describe cysteine on Gold

ReaxFF Parametrization and Validation

Publications useful for these studies:

Checking the behavior of the force fields

The original force field parameters for proteins were extended in this publication:

S. Monti, A. Corozzi, P. Fristrup, K. L. Joshi, Y. K. Shin, P. Oelschlaeger, A. C. T. van Duin, et al.
Exploring the Conformational and Reactive Dynamics of Biomolecules in Solution using an Extended Version of the Glycine Reactive Force Field
Phys. Chem. Chem. Phys. 2013, 15, 15062−15077

Behavior of cysteine in water (in the bulk and at the water/vacuum interface)

A. M. da Silva, A. Mocellin, S. Monti, C. Li, R. R. T. Marinho, A. Medina, H. Ågren, V. Carravetta, A. N. de Brito
Surface-Altered Protonation Studied by Photoelectron Spectroscopy and Reactive Dynamics Simulations.
J. Phys. Chem. Lett. 2015, 6, 807

Nanomechanics of the Disulfide bond in Different Environmental Conditions

S. Keten, C.-C. Chou, A. C. T. van Duin, M. J.Buehler
Tunable nanomechanics of protein disulfide bonds in redox microenvironments
Journal of the Mechanical Behavior of Biomedical Materials 2012, 5, 32-40.

The results shown in this presentation have been published in three recent papers:

S. Monti, V. Carravetta, H. Ågren "Theoretical Study of the Adsorption Mechanism of Cystine on Au(110) in Aqueous Solution"
Small 2016 (accepted for publication)

S. Monti, V. Carravetta, H. Ågren "Simulation of Gold Functionalization with Cysteine by Reactive Molecular Dynamics"
J. Phys. Chem. Lett. 2016, 7, 272−276

S. Monti, V. Carravetta, H. Ågren "Decoration of Gold Nanoparticles with Cysteine in Solution: Reactive Molecular Dynamics Simulations"
Nanoscale, 2016, 8, 12929–12938

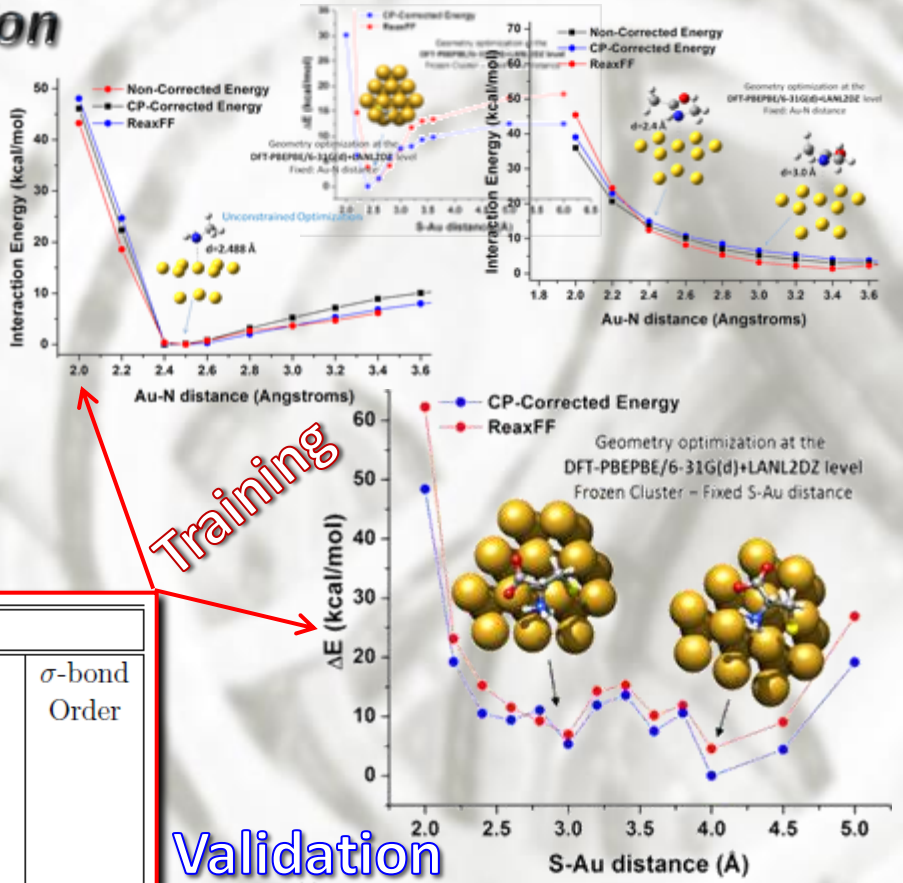
ReaxFF Parametrization and Validation

Starting Data: Force Fields available in the ADF package that have been developed, validated and approved by the authors of ReaxFF, in line with their general policy.

FFs available in the literature: check compatiblity, methods used to determine the parameters.
Quantum Chemistry level and type of calculations

Important: Training Set

Param.	Additions & Changes	ReaxFF Constants				
Bond Au-N Au-S Au-C Au-O Au-H	σ-bond Dissociation Energy	Bond Energy	Overcoord. Penalty	Bond Energy	σ-bond Order	σ-bond Order
	Edis1	pbe1	kov	pbe2	pbo1	pbo2
Off Diag. Au-N Au-S Au-C Au-O Au-H	vdW Dissociation Energy	vdW Radius	vdW Parameter	σ-bond Covalent Radius		
	Ediss	Rvdw	alfa	cov.r		
Val. Angle Au-Au-N Au-N-C	Equil. Angle	First Force const.	Second Force const.	Undercoord.	Ener./bond Order	
	Thetao	ka	kb	pv2	pv3	



ReaxFF Parameters

Param.	Additions & Changes	ReaxFF Constants				
Bond Au-N Au-S Au-C Au-O Au-H	σ-bond Dissociation Energy	Bond Energy	Overcoord. Penalty	Bond Energy	σ-bond Order	σ-bond Order
	Edis1	pbe1	kov	pbe2	pbo1	pbo2
Off Diag. Au-N Au-S Au-C Au-O Au-H	vdW Dissociation Energy	vdW Radius	vdW Parameter	σ-bond Covalent Radius		
	Ediss	Rvdw	alfa	cov.r		
Val. Angle Au-Au-N Au-N-C	Equil. Angle	First Force const.	Second Force const.	Undercoord.	Ener./bond Order	
	Thetao	ka	kb	pv2	pv3	

$$E_{system} = E_{bond} + E_{lp} + E_{over} + E_{under} + E_{val} + E_{pen} + E_{coa} + E_{C2} + E_{tors} + E_{conj} + E_{H\text{-}bond} + E_{vdW} + E_{Coulomb}$$

General and Special Energy Terms

- E_{bond} : bond — directly derived from bond orders — $E_{bond} = -D_e \cdot BO_{ij} \cdot \exp[p_{be,1}(1 - BO_{ij}^{p_{be,1}})]$
- E_{over} : overcoord. — penalty for overcoordinating atoms — $E_{over} = p_{over} \cdot \Delta_i \cdot \left(\frac{1}{1 + \exp(\lambda_6 \cdot \Delta_i)}\right)$
- E_{val} : angle strain — equilibrium angle depends on bond order central atom — $E_{val} = f_7(BO_{ij}) \cdot f_7(BO_{jk}) \cdot f_8(\Delta_j) \cdot \{k_a - k_a \exp[-k_b(\Theta_o - \Theta_{ijk})^2]\}$
- E_{tors} : torsion — bond-order dependent V2-term — $E_{tors} = f_{10}(BO_{ij}, BO_{jk}, BO_{kl}) \cdot \sin\Theta_{ijk} \cdot \sin\Theta_{jkl} \left[\frac{1}{2}V_1 \cdot \exp(p_1(BO_{jk} - 3 + f_{11}(\Delta_j, \Delta_k))^2] \cdot (1 - \cos 2\omega_{ijkl}) + \frac{1}{2}V_3 \cdot (1 + \cos 3\omega_{ijkl})\right]$
- E_{vdW} : van der Waals — between every atom — $E_{vdWaals} = D_{ij} \cdot \left\{\exp\left[\alpha_{ij} \cdot \left(1 - \frac{f_{13}(r_{ij})}{r_{vdW}}\right)\right] - 2\exp\left[\frac{1}{2} \cdot \alpha_{ij} \cdot \left(1 - \frac{f_{13}(r_{ij})}{r_{vdW}}\right)\right]\right\}$
- $E_{Coulomb}$: Coulomb — between every atom; polarizable charges updated every iteration — $E_{Coulomb} = C \cdot \frac{q_i \cdot q_j}{[r_{ij}^3 + (1/\gamma_{ij})^3]^{1/3}}$

Force field identifier

General

Parameter identifiers

Atom

Bond

Off-diagonal

Angle

Torsion

H-bond

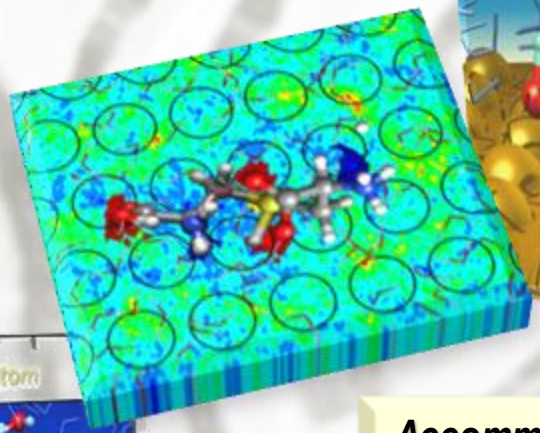
```
Reactive MD-force field: Hydrocarbon parameters
 39       ! Number of general parameters
  50.0000 !Overcoordination parameter
   9.8407 !Overcoordination parameter
  21.2839 !Valency angle conjugation parameter
   3.0000 !Triple bond stabilization parameter
   6.5000 !Triple bond stabilization parameter
   1.0000 !Not used
   0.9782 !Undercoordination parameter
   1.0250 !Triple bond stabilization parameter
   6.3452 !Undercoordination parameter
  11.6274 !Undercoordination parameter
   0.0000 !Triple bond stabilization energy
   0.0000 !Lower Taper-radius
  10.0000 !Upper Taper-radius
   2.8793 !Not used
  33.8667 !Valency undercoordination
  88.6186 !Valency angle/lone pair parameter
   1.0563 !Valency angle
   2.0384 !Valency angle parameter
   6.1431 !Not used
   7.5203 !Double bond/angle parameter
   0.3989 !Double bond/angle parameter: overcoord
   3.9954 !Double bond/angle parameter: overcoord
  -2.4837 !Not used
   4.7120 !Torsion/BO parameter
  10.0000 !Torsion overcoordination
   2.3170 !Torsion overcoordination
  -1.2635 !Conjugation 0 (not used)
   2.1645 !Conjugation
   1.4553 !vdWaals shielding
   0.1000 !Cutoff for bond order (*100)
   2.8921 !Valency angle conjugation parameter
   7.1783 !Overcoordination parameter
   1.4473 !Overcoordination parameter
   3.1353 !Valency/lone pair parameter
   0.5000 !Not used
  20.0000 !Not used
   5.0000 !Molecular energy (not used)
   0.0000 !Molecular energy (not used)
   1.6052 !Valency angle conjugation parameter
 2
      cov.r; valency;  a.m;      Rvdw;   Evdw;   gammaEEM;  cov.r2;  #el
      alfa;  gammavdW; valency; Eunder; n.u.;   chiEEM;    etaEEM;  n.u.
      cov r3;Elp;      Heat inc.;13BO1; 13BO2;  13BO3;     n.u.;    n.u.
      ov/un; val1;     n.u.;     val3;  vval4;  n.u.;      n.u.;    n.u.
C     1.3826   4.0000  12.0000   2.0195   0.0763   0.8712   1.2360   4.0000
     10.6359   1.9232   4.0000  40.5154   0.0000   5.7254   6.9235   0.0000
      1.1663   0.0000 200.0498   6.1551  28.6991  12.1086   0.0000   0.0000
    -14.1953   3.5288   0.0000   6.2998   2.9663   0.0000   0.0000   0.0000
H     0.6510   1.0000   1.0080   1.7693   0.0244   0.7625  -0.1000   1.0000
     10.0482   5.2587   1.0000   0.0000   0.0000   3.8196   9.8832   1.0000
     -0.1000   0.0000  65.0500   3.7647   1.0000   0.0000   0.0000   0.0000
    -13.3669   3.6815   0.0000   6.2998   2.8793   0.0000   0.0000   0.0000
 3
      Edis1;    Edis2;    Edis3;    pbe1;    pbo5;    13corr;  pbo6;    kov
      pbe2;     pbo3;     pbo4;     n.u.;    pbo1;    pbo2;    ovcorr;  n.u.
 1  1 152.0140 104.0507  72.1693   0.2447  -0.7132   1.0000  23.5135   0.3545
       0.1152  -0.2069   9.2317   1.0000  -0.1042   5.9159   1.0000
 1  2 174.2967   0.0000   0.0000  -0.5193   0.0000   1.0000   6.0000   0.4401
      18.9231   1.0000   0.0000   1.0000  -0.0099   8.2733   0.0000
 2  2 177.8312   0.0000   0.0000  -0.3029   0.0000   1.0000   6.0000   0.6891
      10.6518   1.0000   0.0000   1.0000  -0.0191   5.4288   0.0000
 1
      Evdw;     Rvdw;     alfa;     cov.r;   cov.r2;  cov.r3
 1  2   0.0404   1.8583  10.3804   1.0376  -1.0000  -1.0000
 3
      theta0;   ka;       kb;       pconj;   pv2;     kpenal;  pv3
 1  1  1  70.2140  14.0458   2.0508   0.0000   0.0000  35.9933   1.0400
 1  1  2  71.6289  18.4967   8.4619   0.0000   0.0000   0.0000   1.0400
 2  1  2  72.7374  18.0638   2.9517   0.0000   0.2000   0.0000   1.0400
 4
      V1;       V2;       V3;       V2(BO);  vconj;   n.u;     n.u.
 1  1  1  1   0.0000  28.8256   0.1796  -4.6957  -1.3130   0.0000   0.0000
 1  1  1  2   0.0000  32.8083   0.4536  -4.6087  -1.7236   0.0000   0.0000
 2  1  1  2   0.0000  36.7455   0.3087  -4.7435  -0.7311   0.0000   0.0000
 0  1  2  0   0.0000  00.0000   0.1000  -4.7435   0.0000   0.0000   0.0000
 1
      Rhb;      Dehb;     vhb1;     vhb2
 1  2  1   2.0347   0.0000   4.9076   4.2357
```

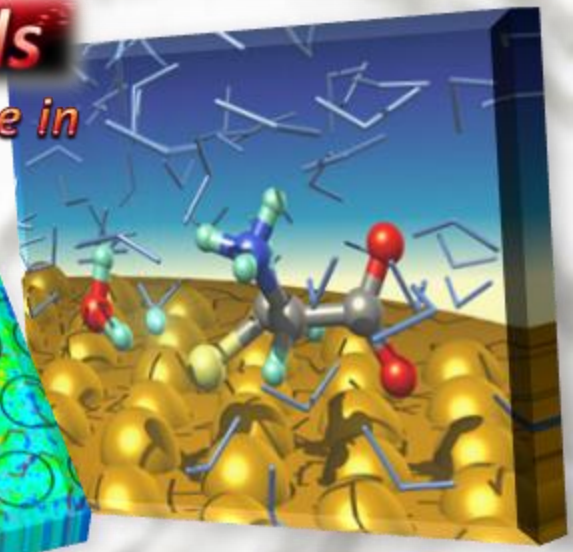
ReaxFF MDs Results

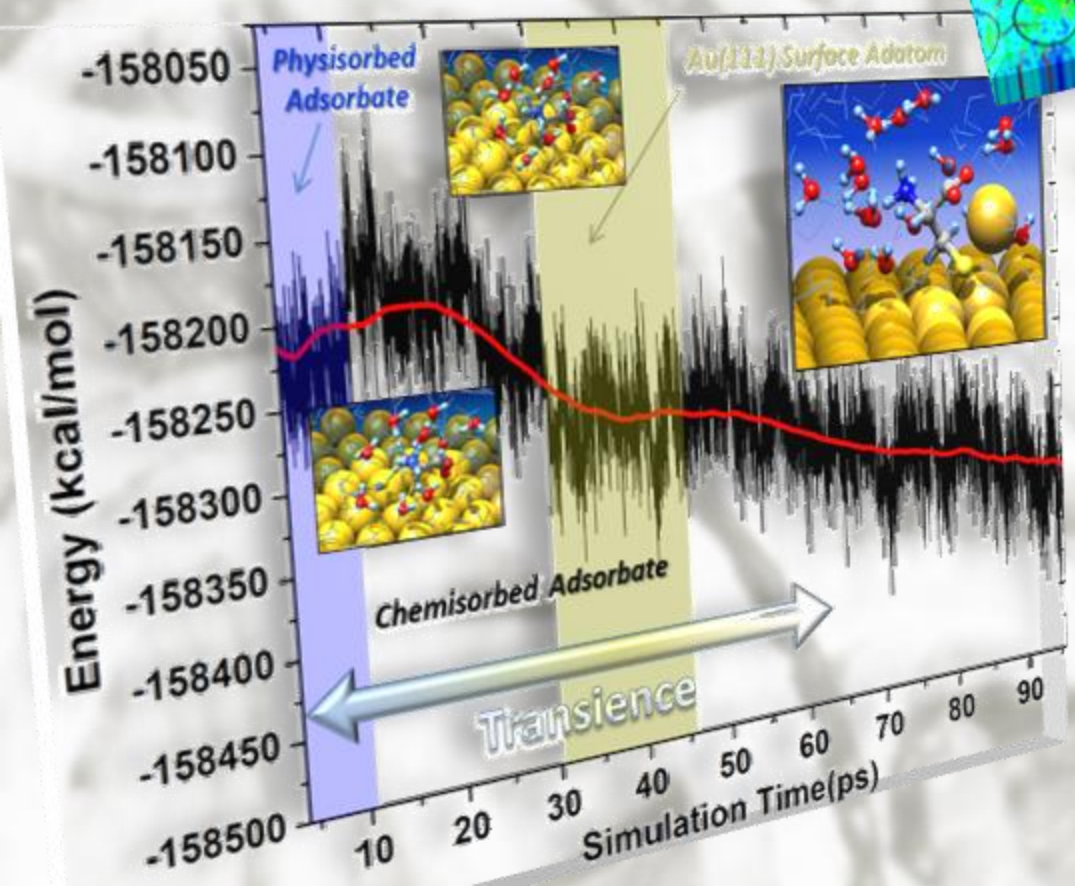
Force Field in Action: Smaller Models

MDs of the adsorption of Cysteine on the Au(111) Interface in Solution: physisorption and chemisorption

Model: one cysteine , 303 wats,
Au(111) slab (ten layers with 90 Au atoms each)
Simulation box = 26x25x70 Å^3
NVT-MDs at T=300 K, P=1 atm (ADF/ReaxFF)
Equilibration = 25 ps (physisorption),
Production = 100 ps, step = 0.25 fs – sampling: 0.025 ps

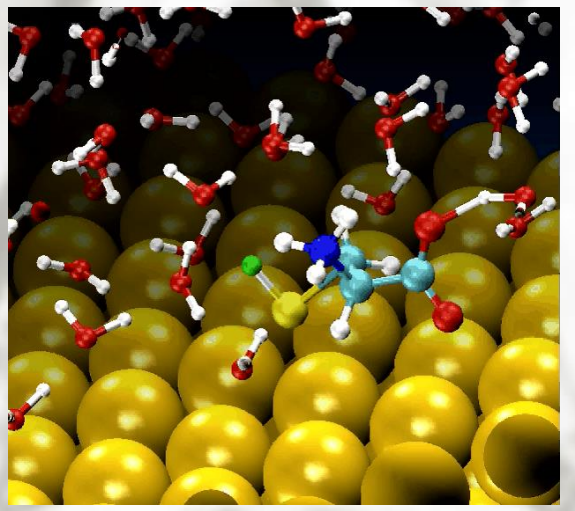


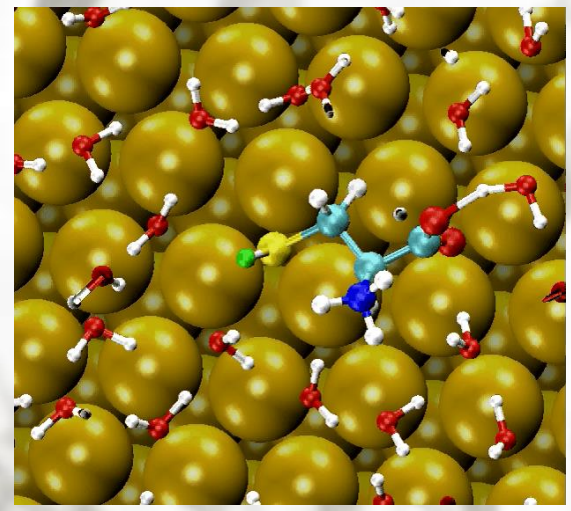


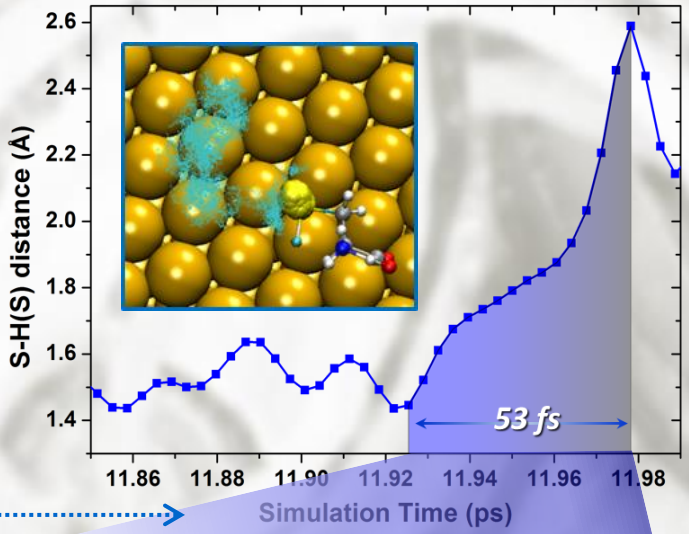


Accommodation of CYS on the surface

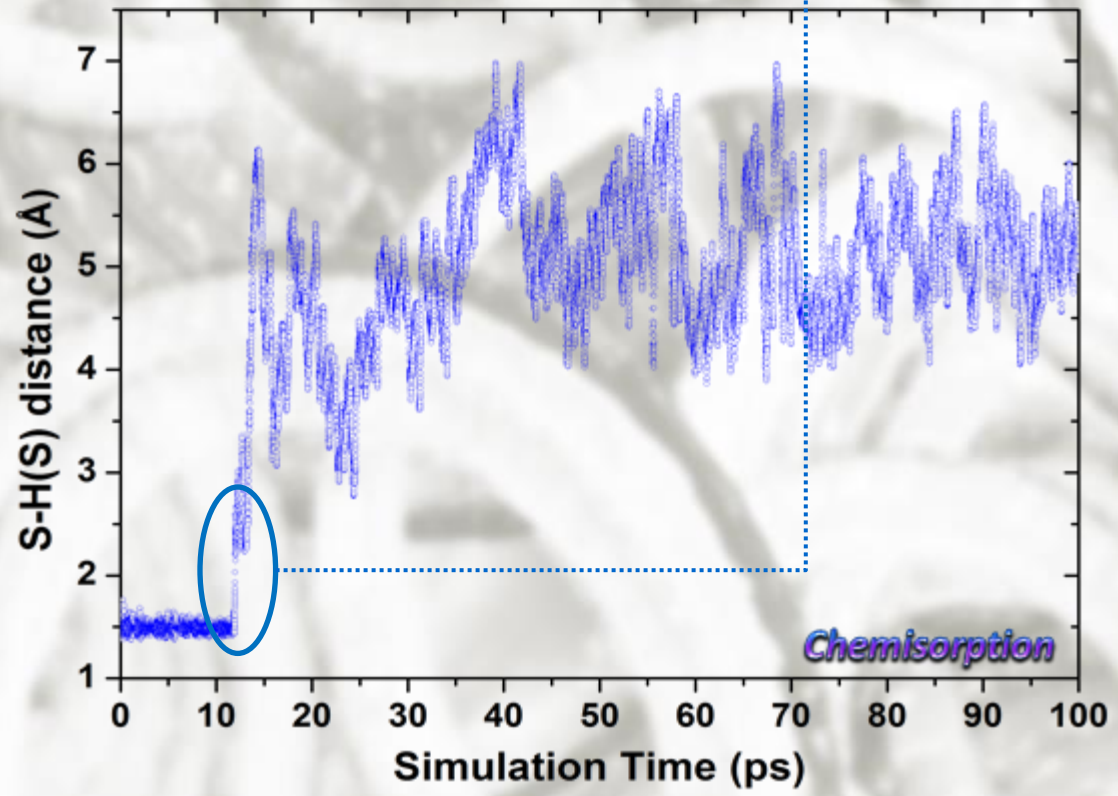
- Riorientation of Os towards gold
  - Attraction and Capture of an Au atom – adatom
- ***Physisorption*** of SH
  - Relocation of S to improve the connection
    - Release of HS to WAT
      - ***Chemisorption***: Au-S bond

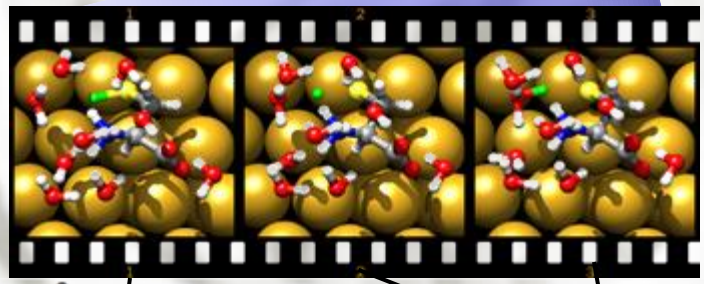


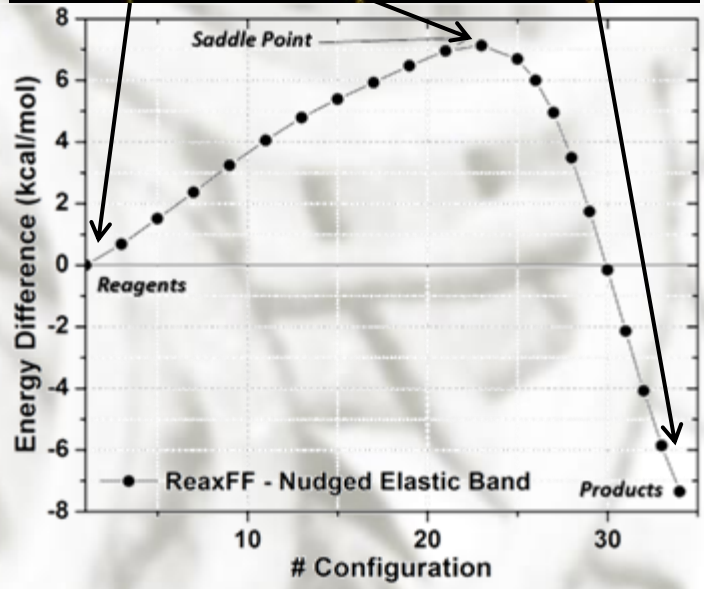


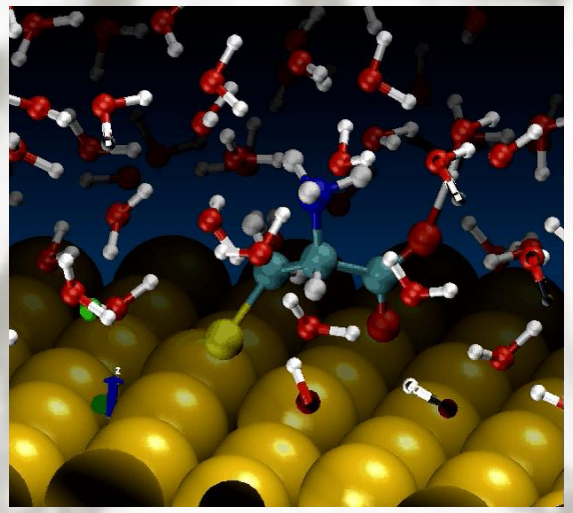


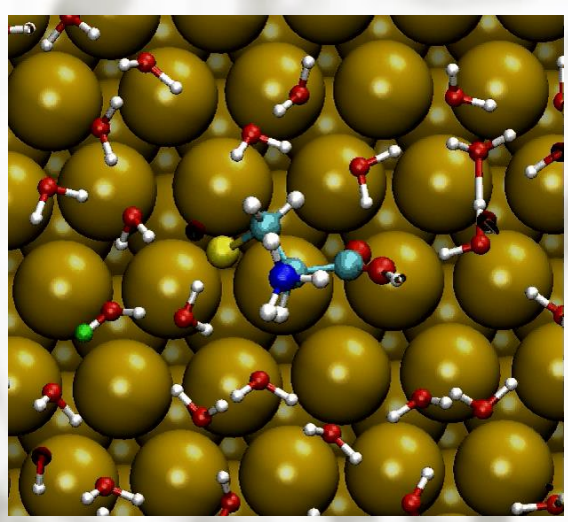
SH bond breaking: release of HS to water

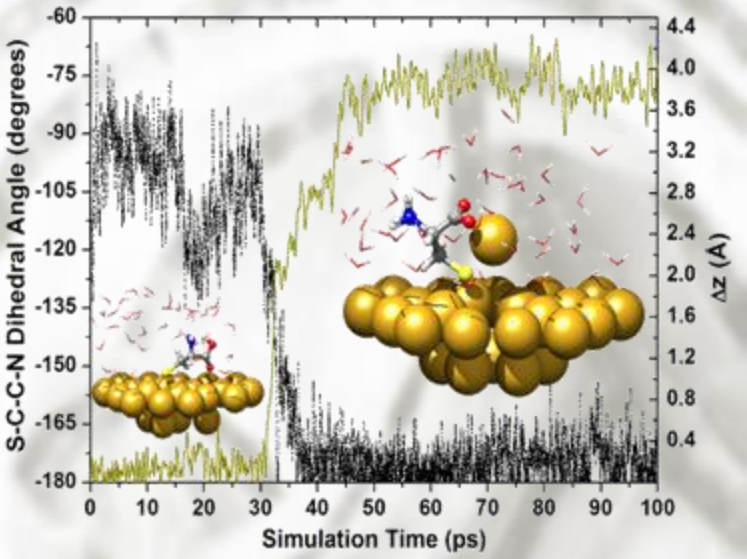




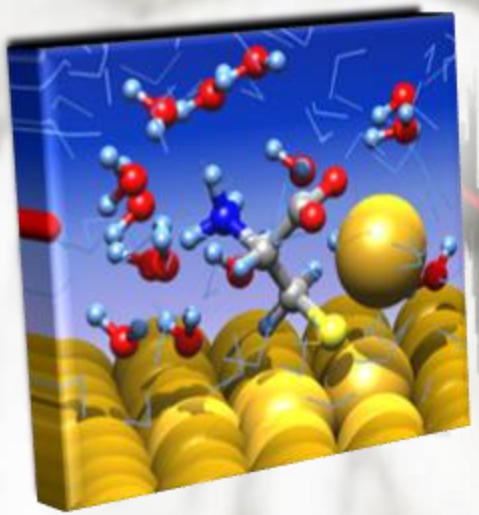


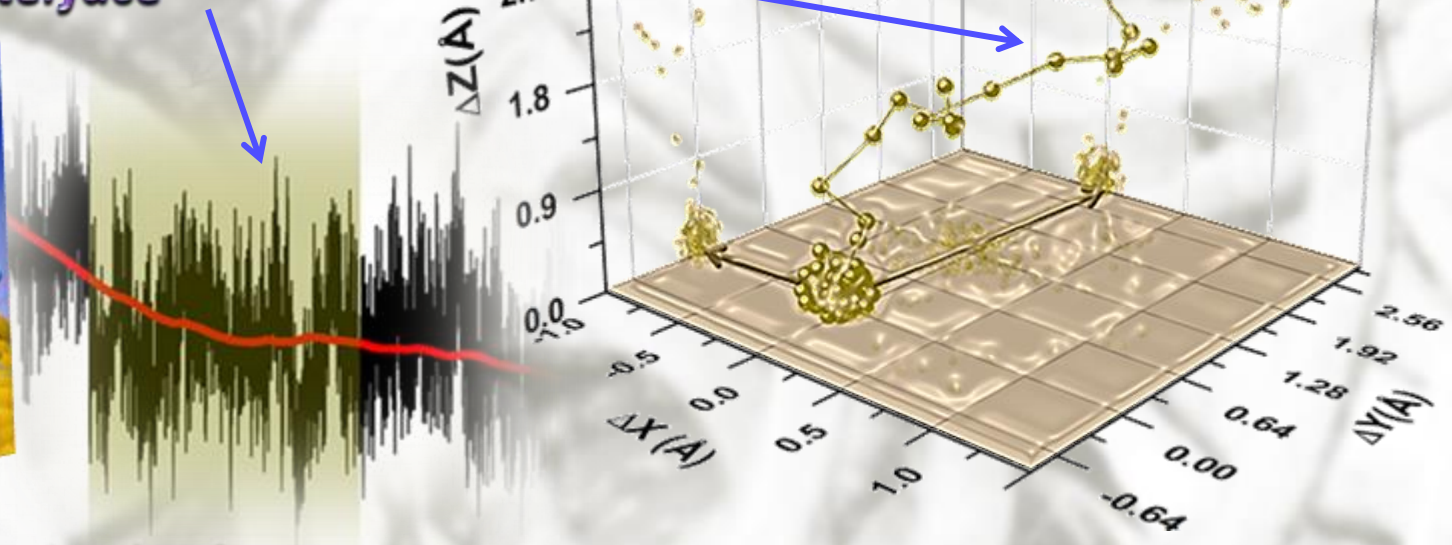




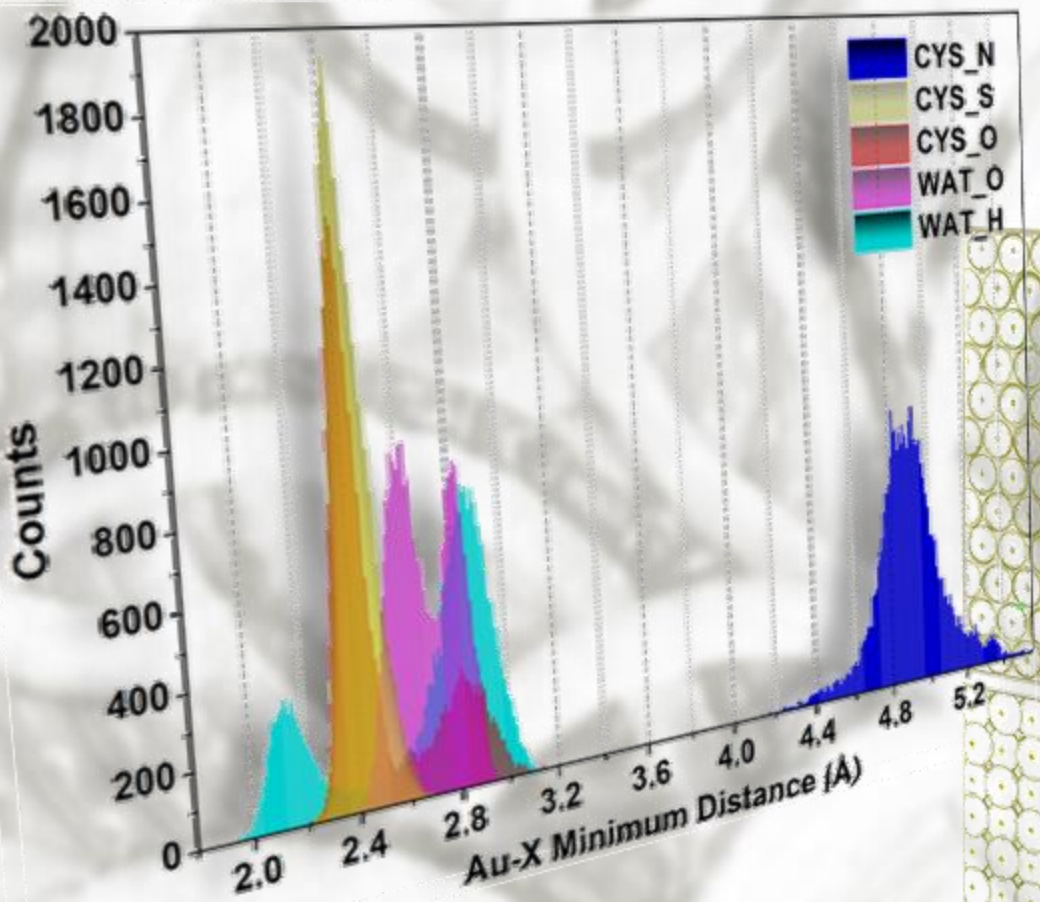


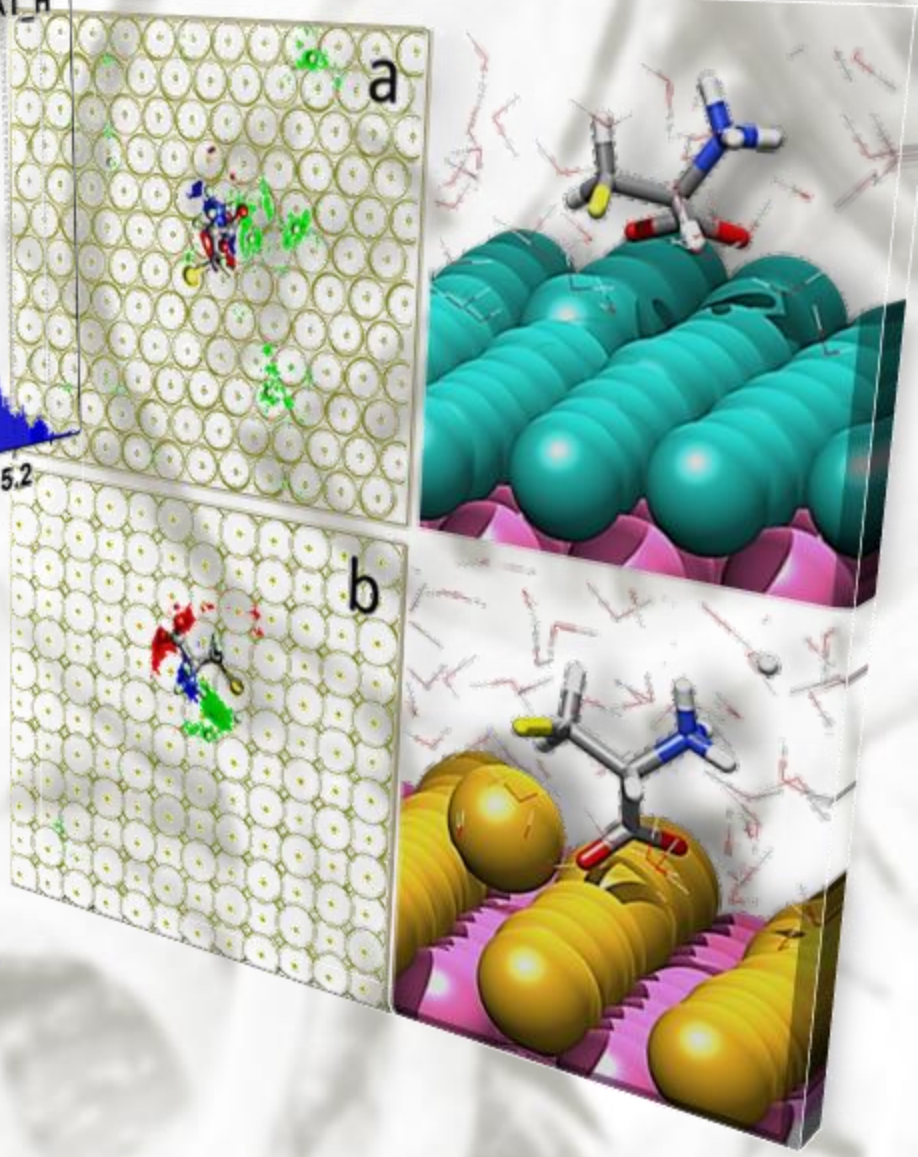
Formation of an adatom on Au(111) and stabiliaztion of the molecule on the support. Distance travelled by the adatom in relation to its initial location on the interface





Stable connection through S and O atoms. NH_3 remains in solution

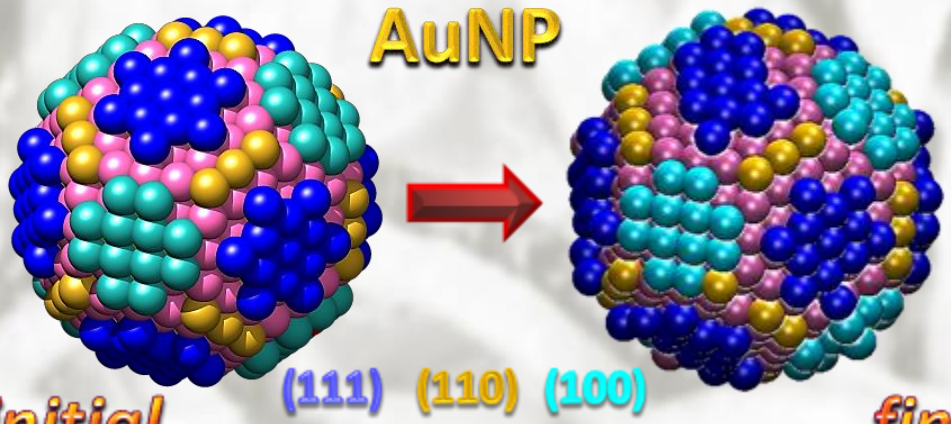


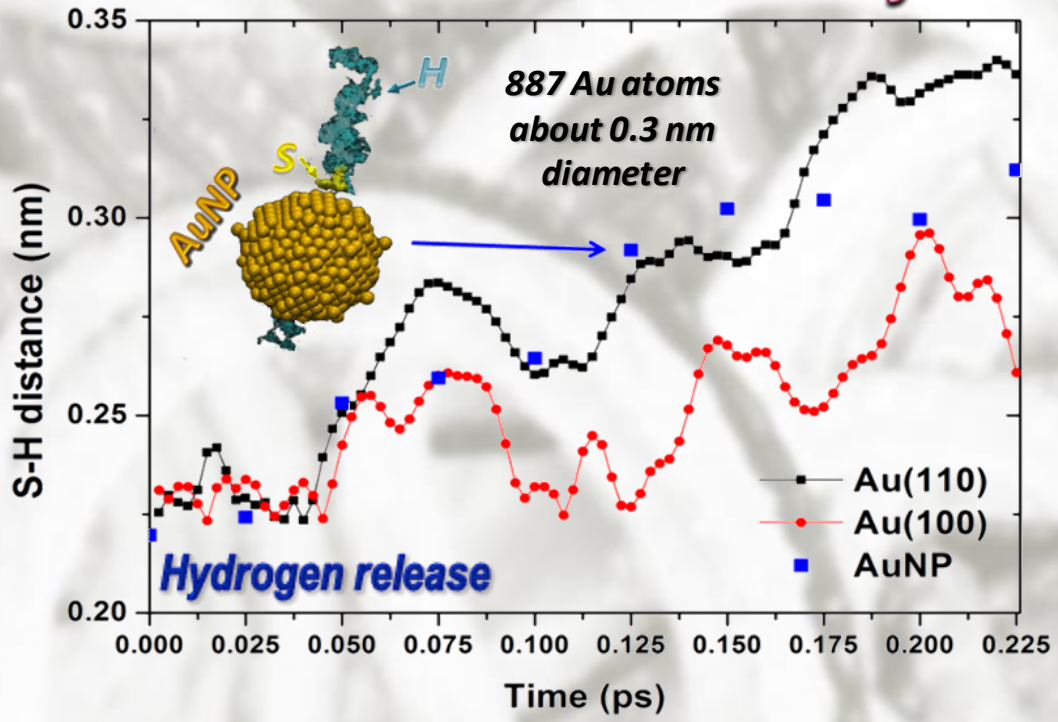


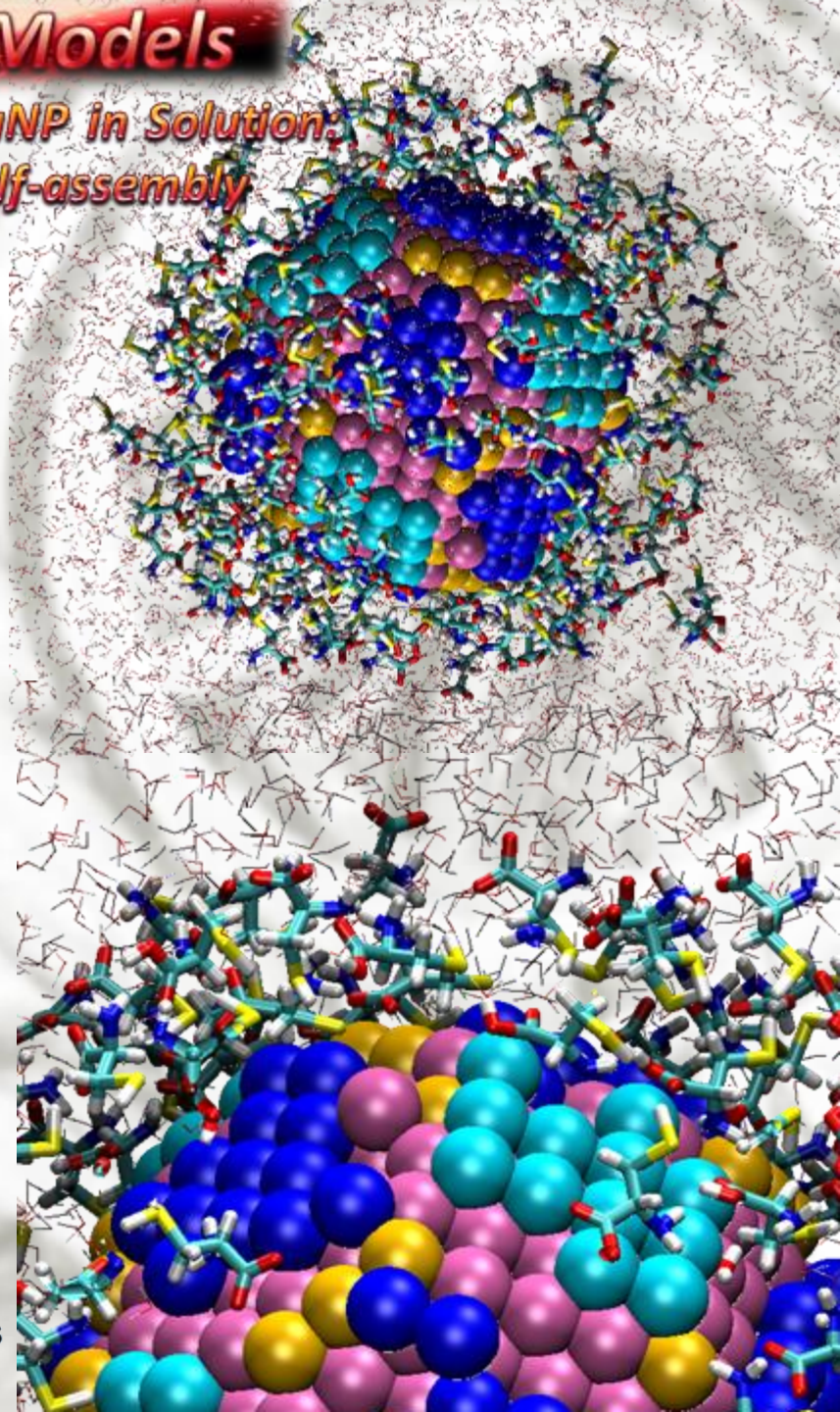
Similar behavior when cysteine is adsorbed on the Au(100) (a) and Au(110) (b) surfaces. Formation of an adatom was noticed as well

Force Field in Action: Larger Models

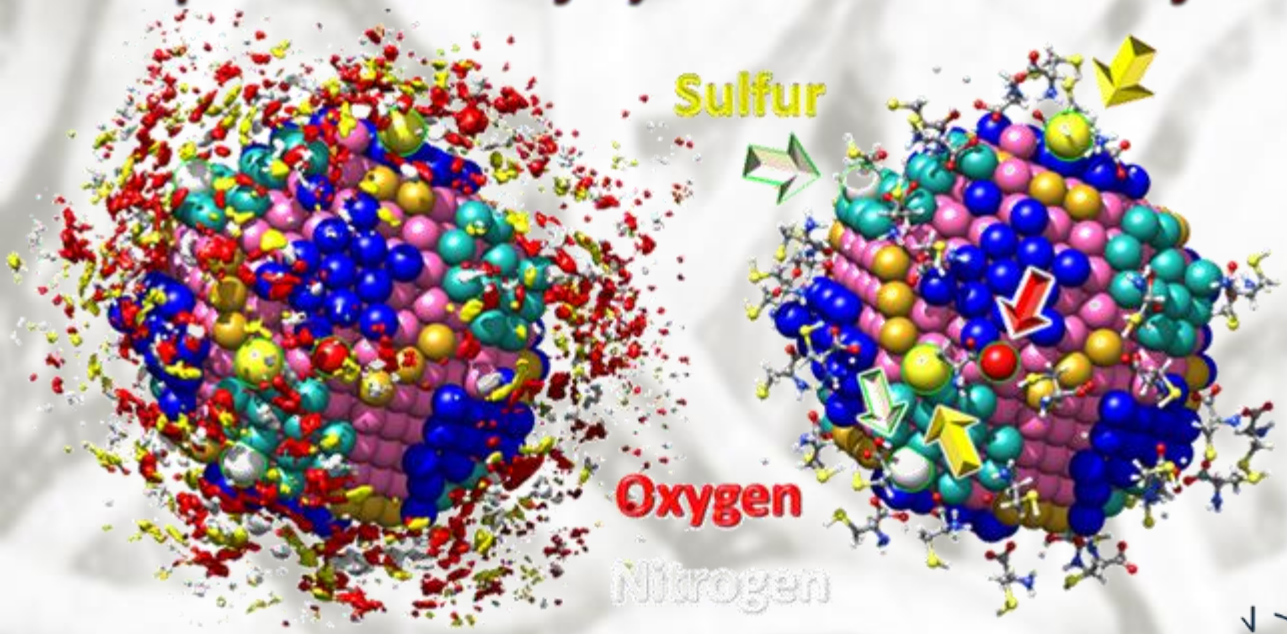
MDs of the adsorption of Cysteines on an AuNP in Solution: Physisorption, Chemisorption and molecular self-assembly

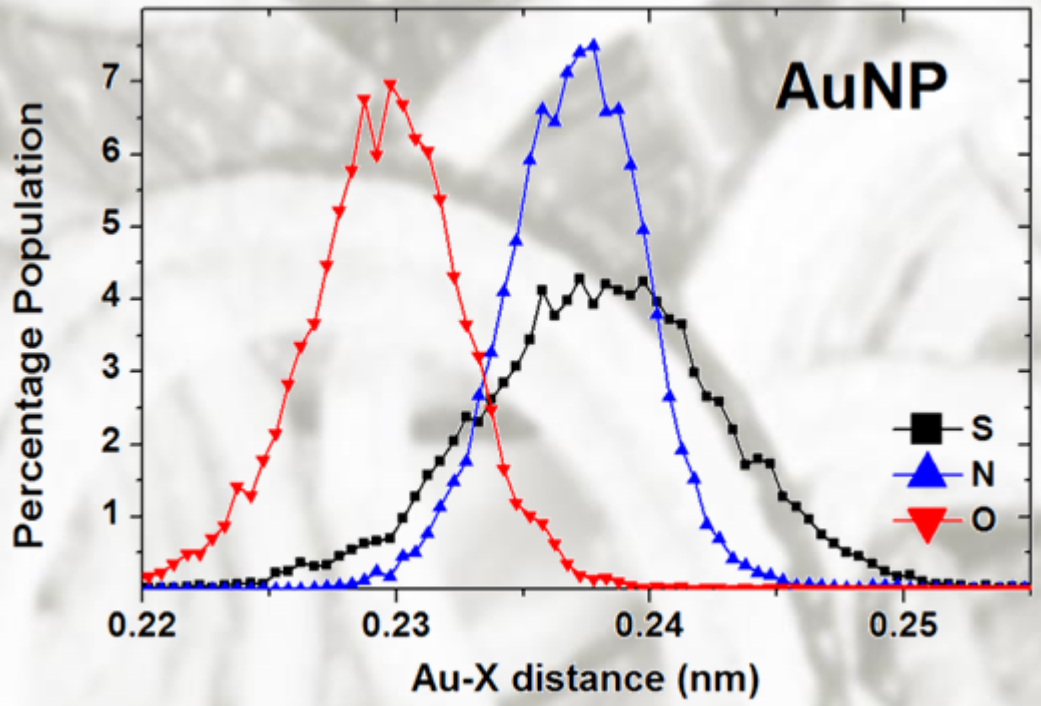


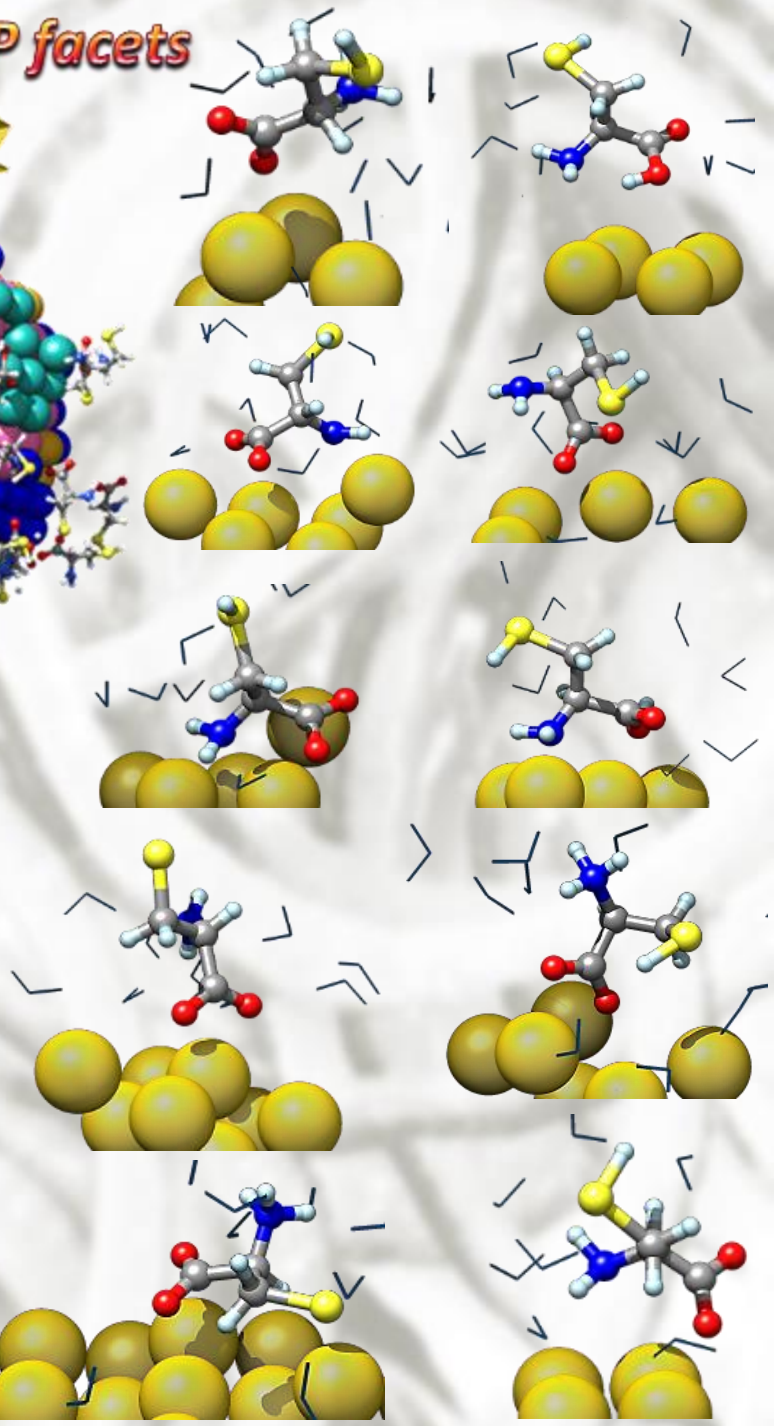




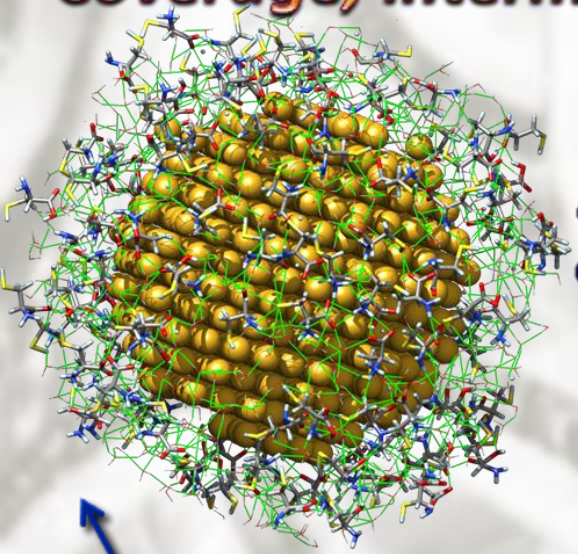
Adsorption Modes of Cysteine on the AuNP facets

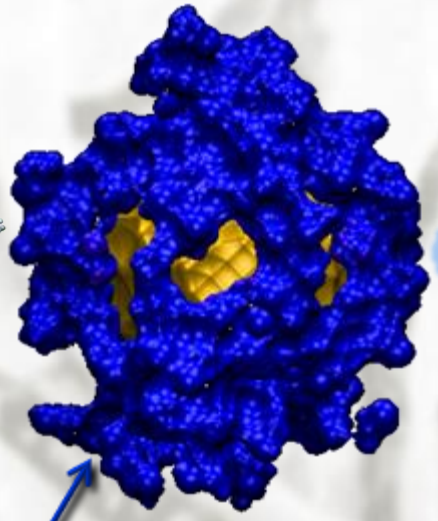


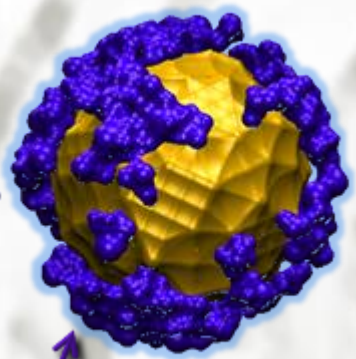


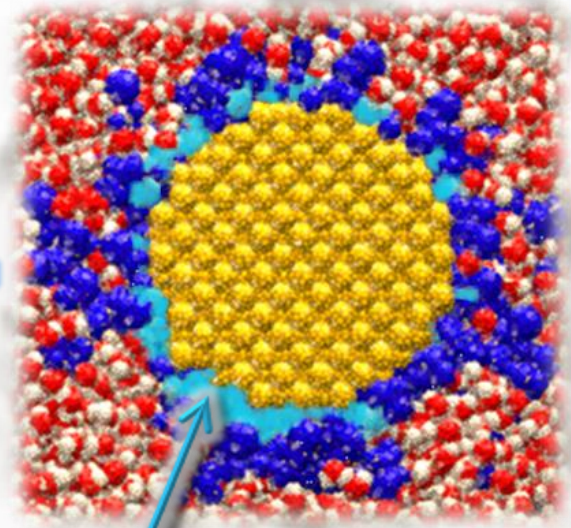


Coverage, intermolecular connections and AuNP solvation





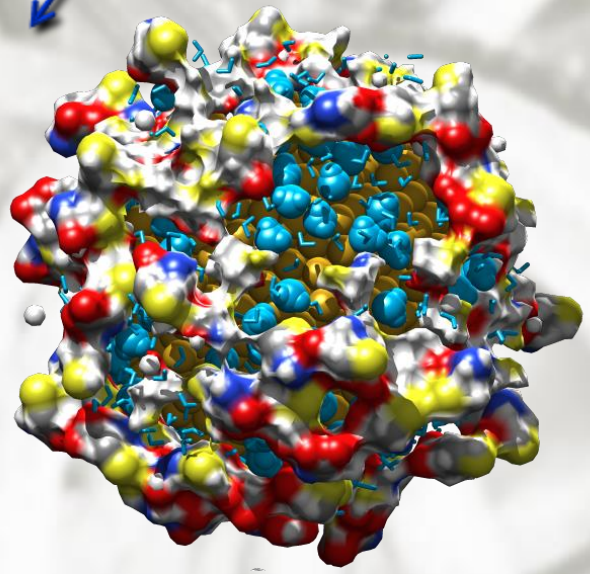


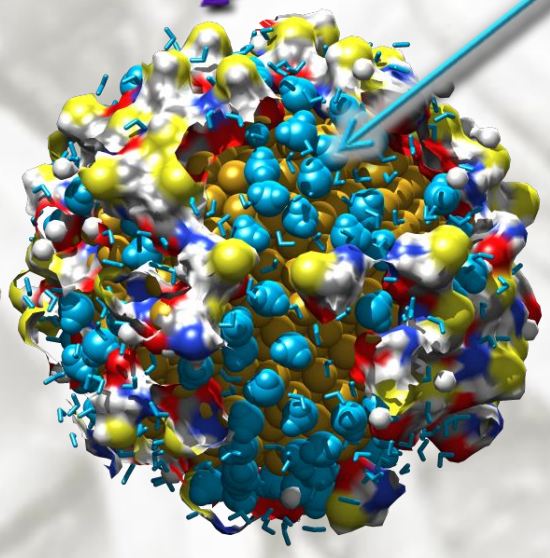


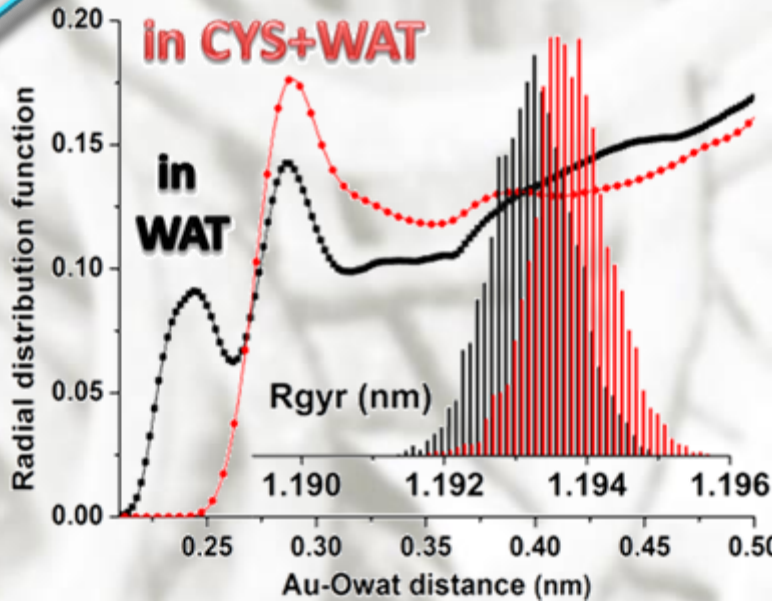
all cysteine molecules around the AuNP

adsorbates within 0.35 nm of the AuNP

Waters in contact with the AuNP trapped by the cysteine network

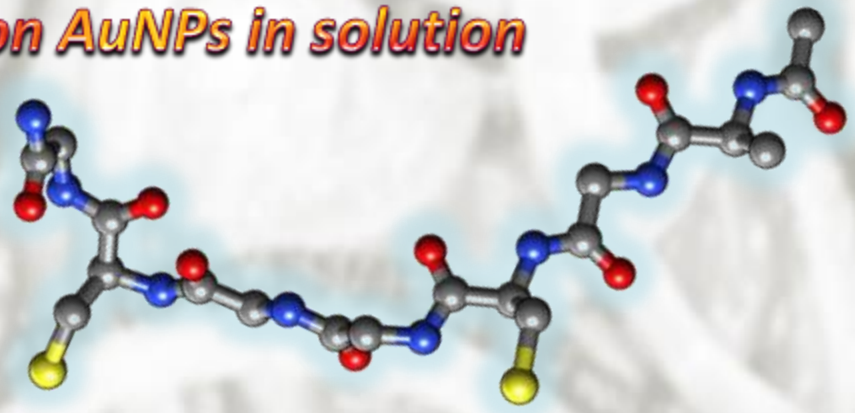




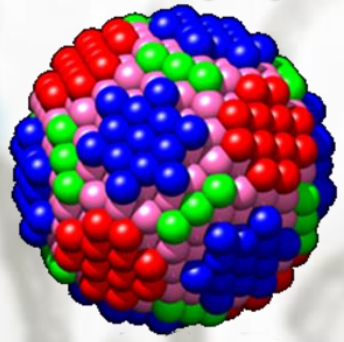


Force Field in Action: Larger Models

MDs of the adsorption of Cysteine-based peptides on AuNPs in solution



Ace-ALA-GLY-**CYS**-GLY-GLY-**CYS**-GLY-NH_2

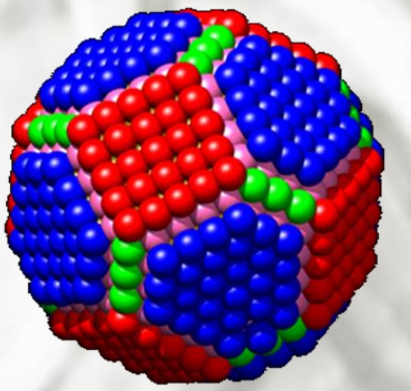


887

d = 2 nm

Au{110}
Au{111}
Au{100}

Bigger AuNP

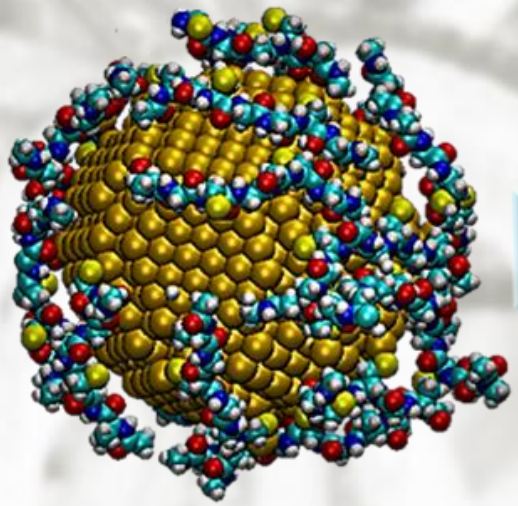


1505

d = 3 nm

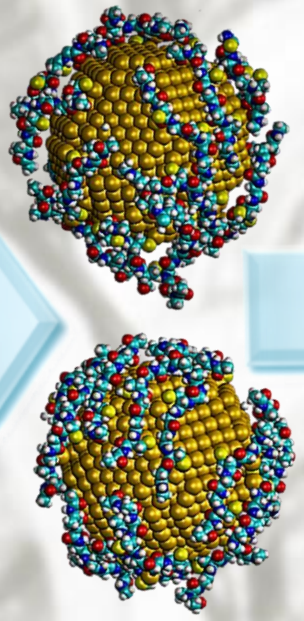
Molecular Dynamics Simulations in the NPT ensemble at T≈ 310 K

Au(1505) + 24 peptide chains (1512 atoms)

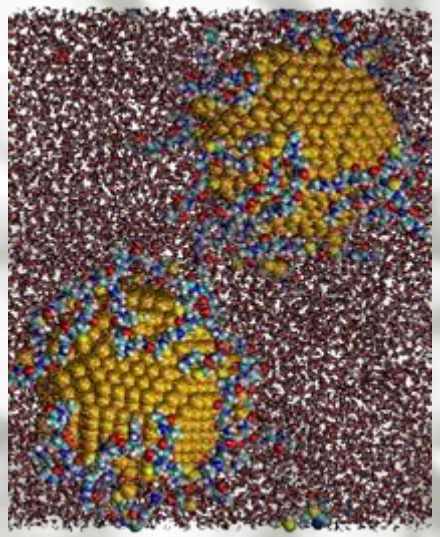


3017 atoms

6034 atoms



+ 17300 waters

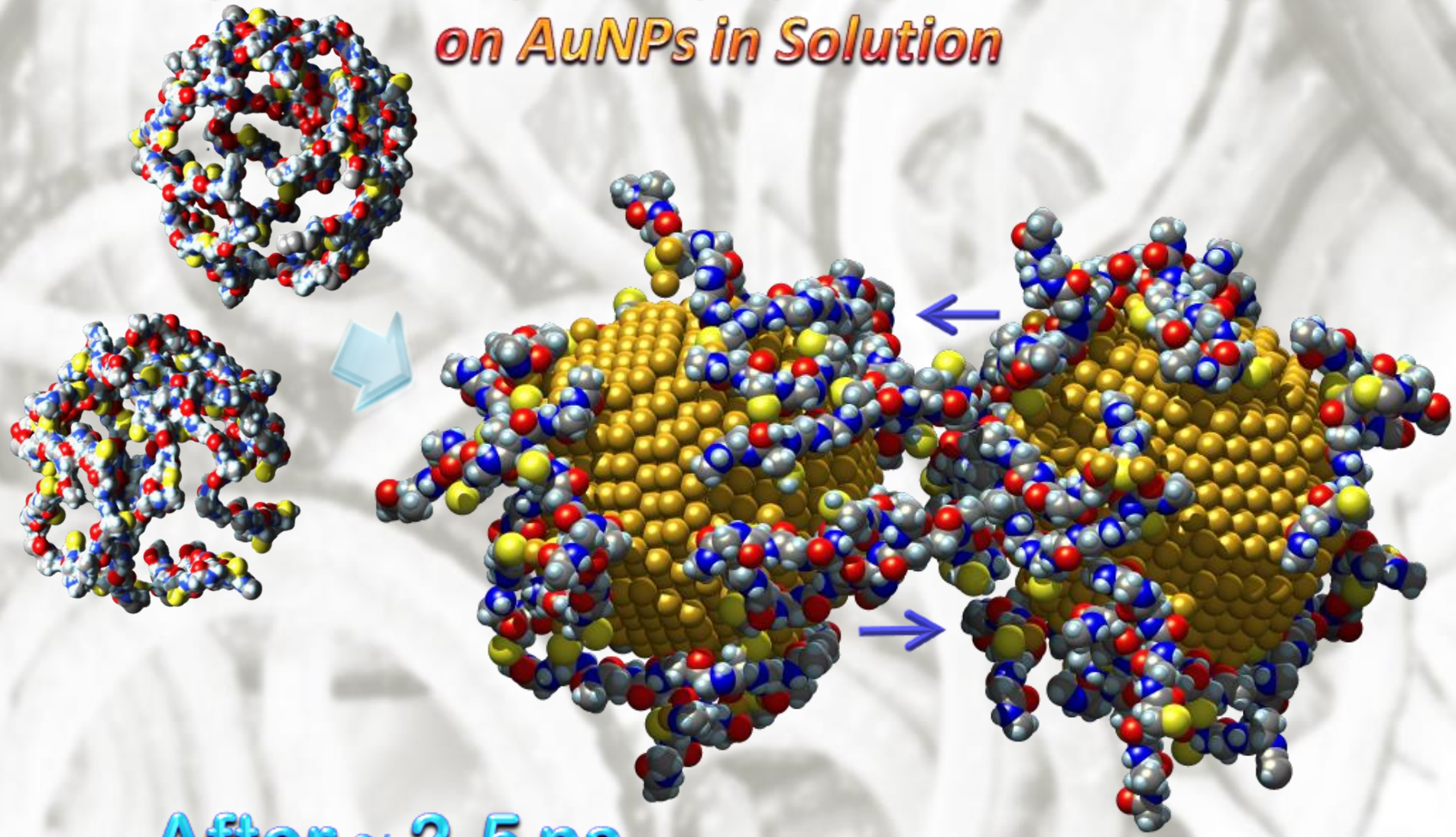


≈ 58000 atoms

Partially covered AuNPs (adatoms) Snapshot extracted after ≈ 1.0 ns

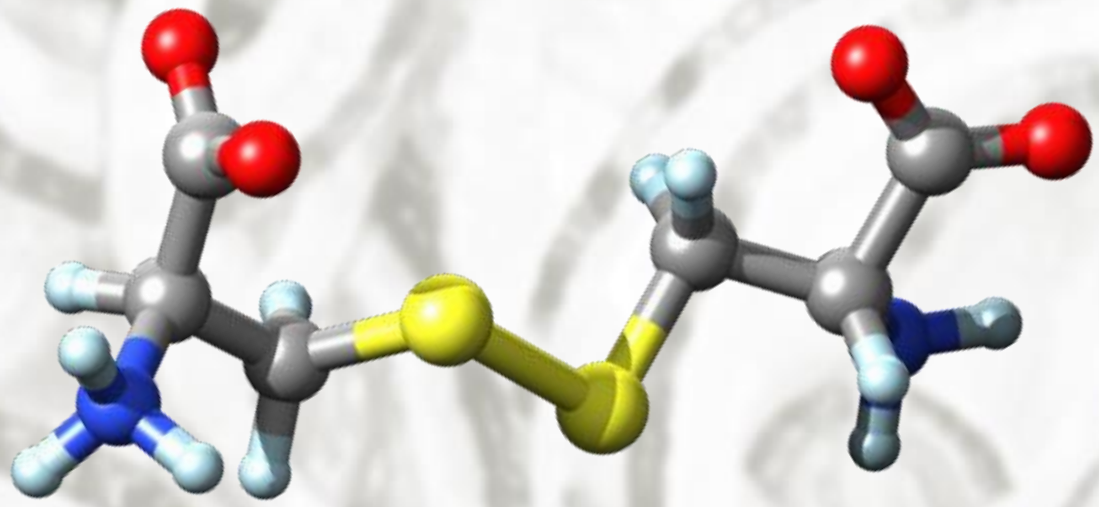
Force Field in Action: Larger Models

MDs of the Adsorption of Cysteine-based Peptides on AuNPs in Solution

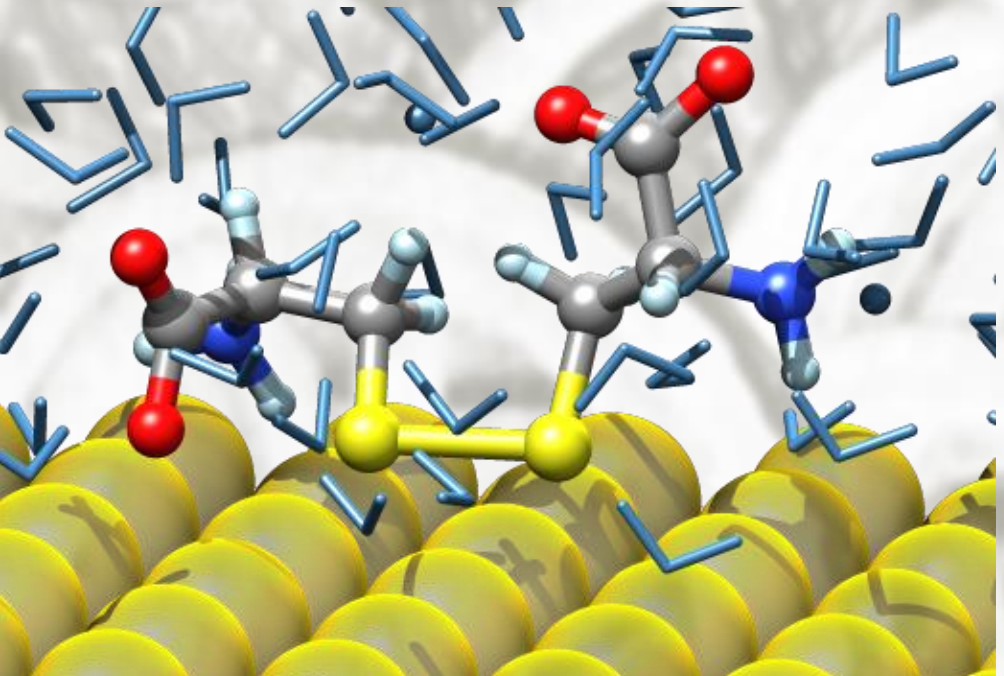


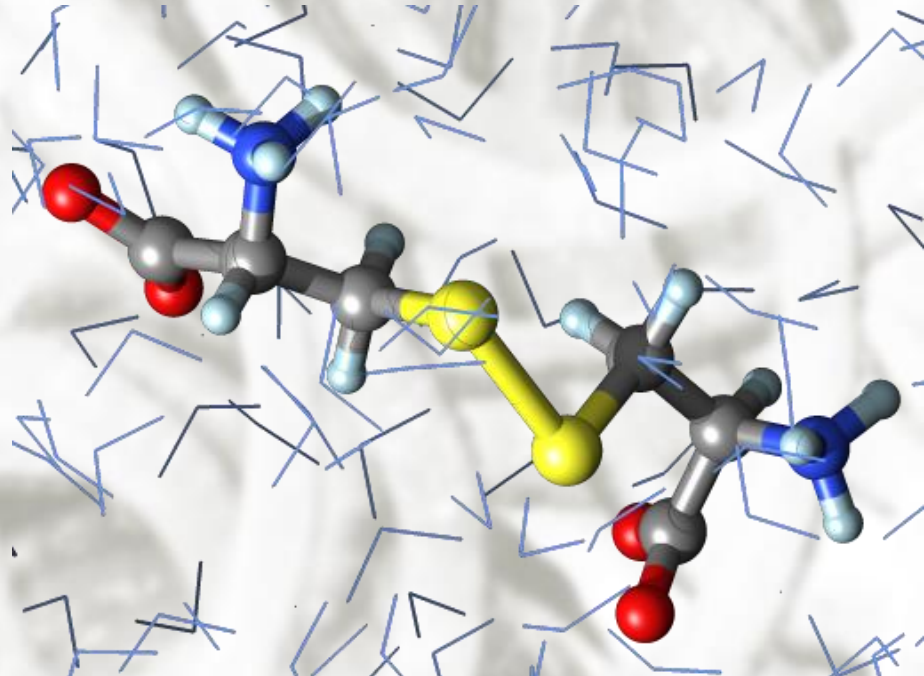
After ≈ 2.5 ns

work in progress ...



Cysteine's twin sister: Cystine





Cystine on Au(110): Single Adsorption

Parallel Orientation

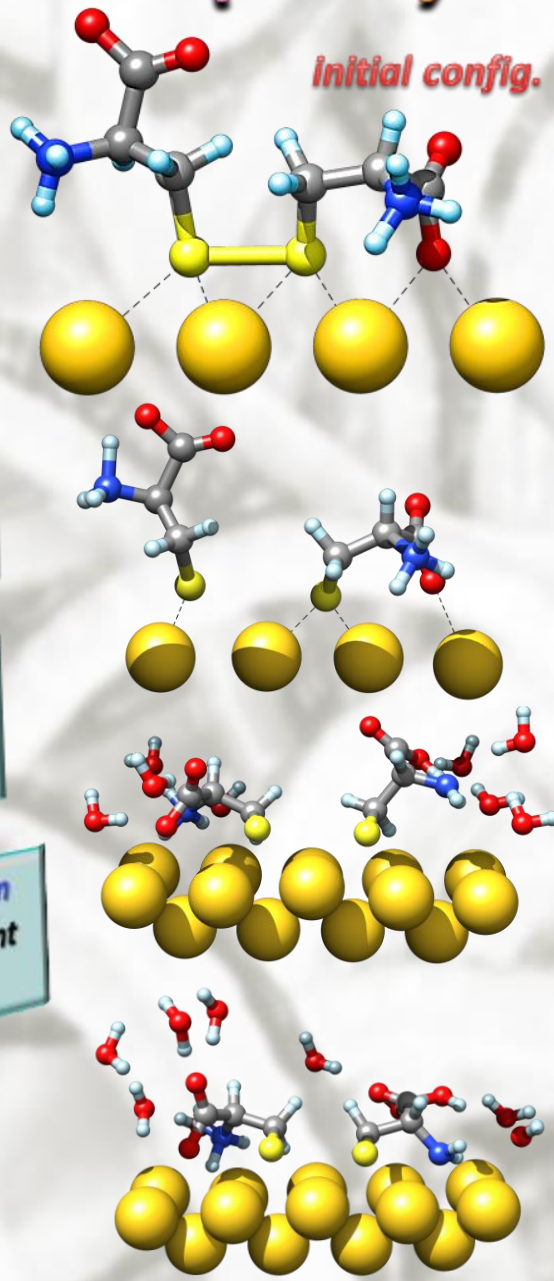
Disulfide bond breaking

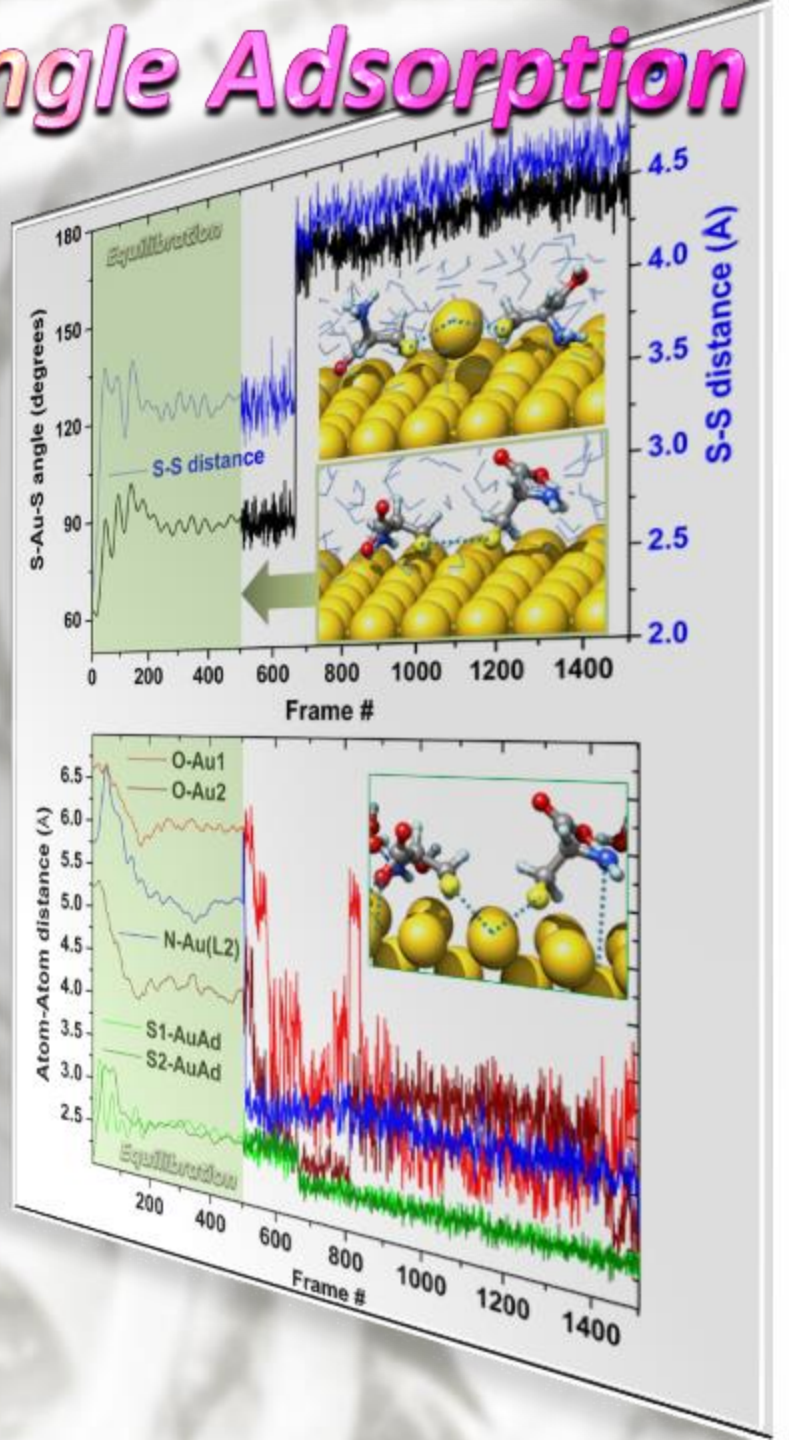
Relocation of the sulfur atoms to improve the connection

Relocation of the Carboxyl Oxygens to improve adsorption

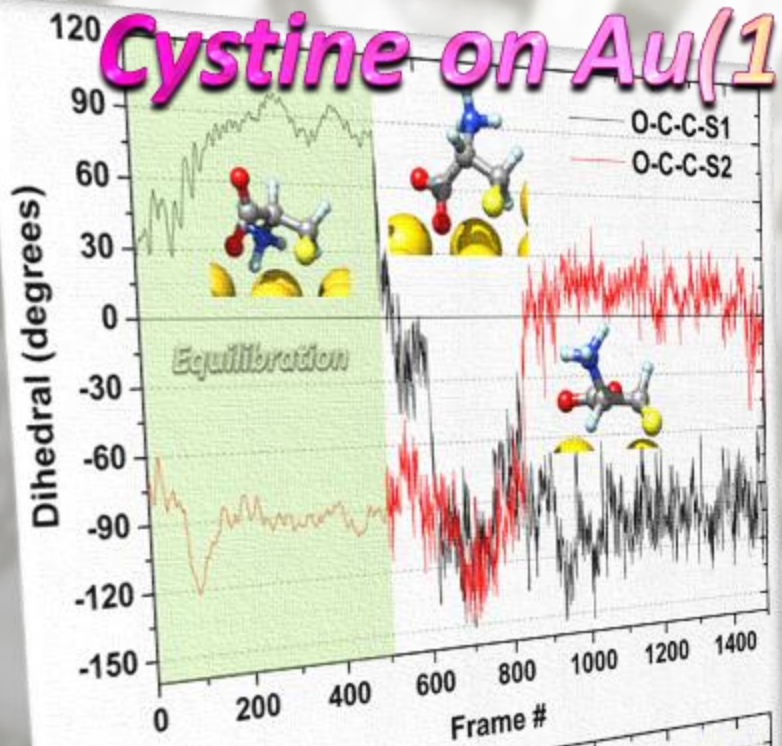
Coordination of the Nitrogen atom to the support (covalent connection to gold)

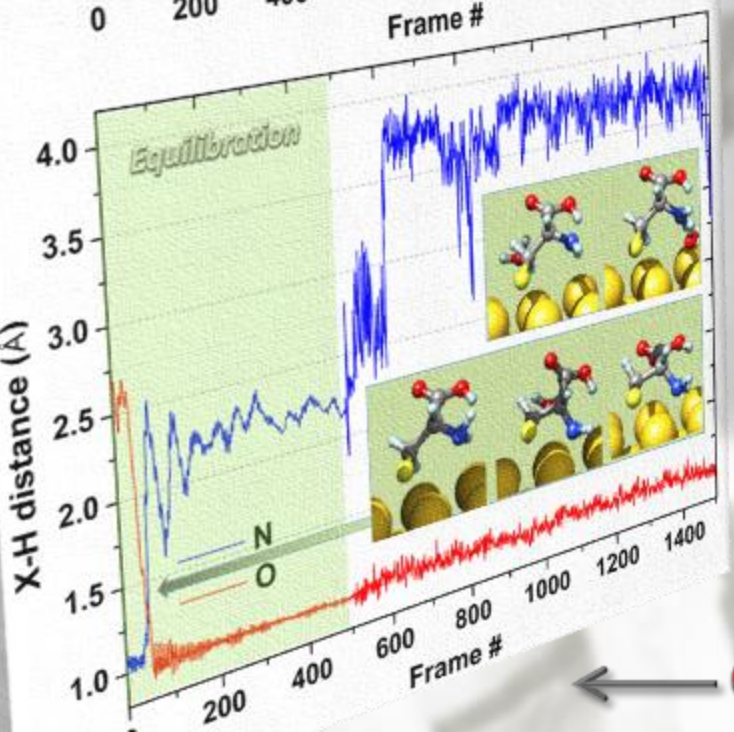
Staple formation



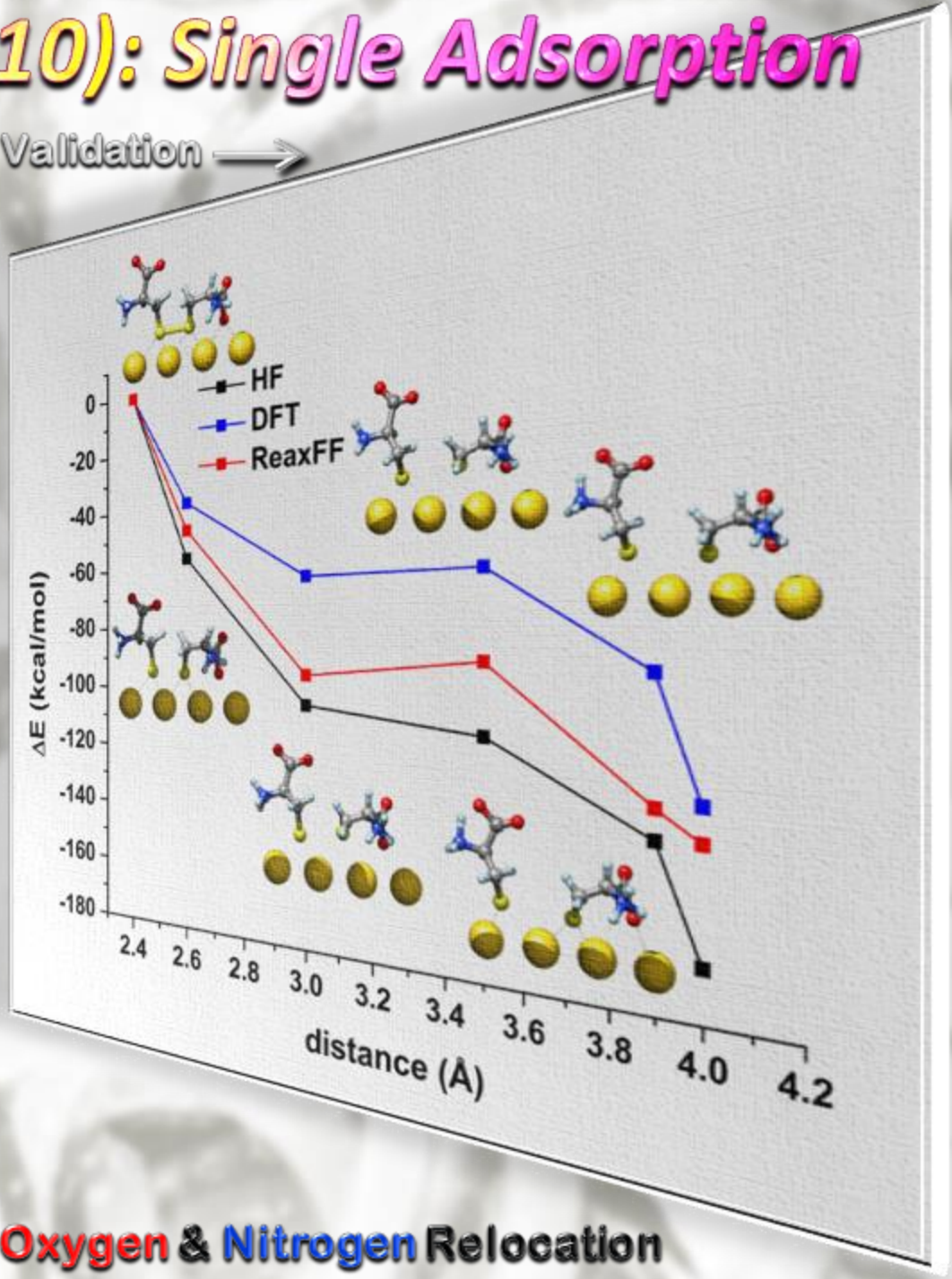


Cystine on Au(110): Single Adsorption

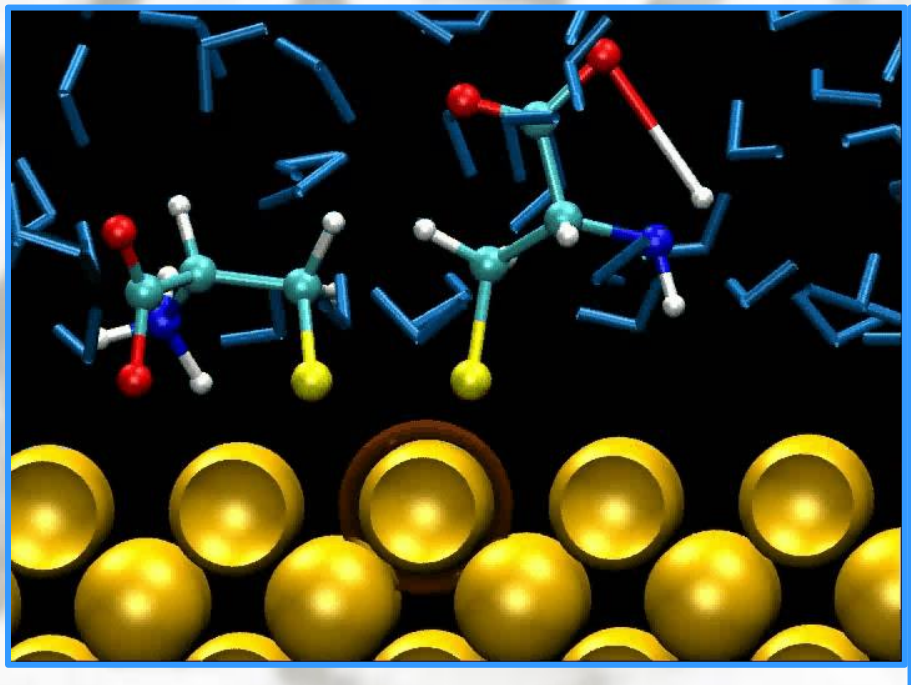


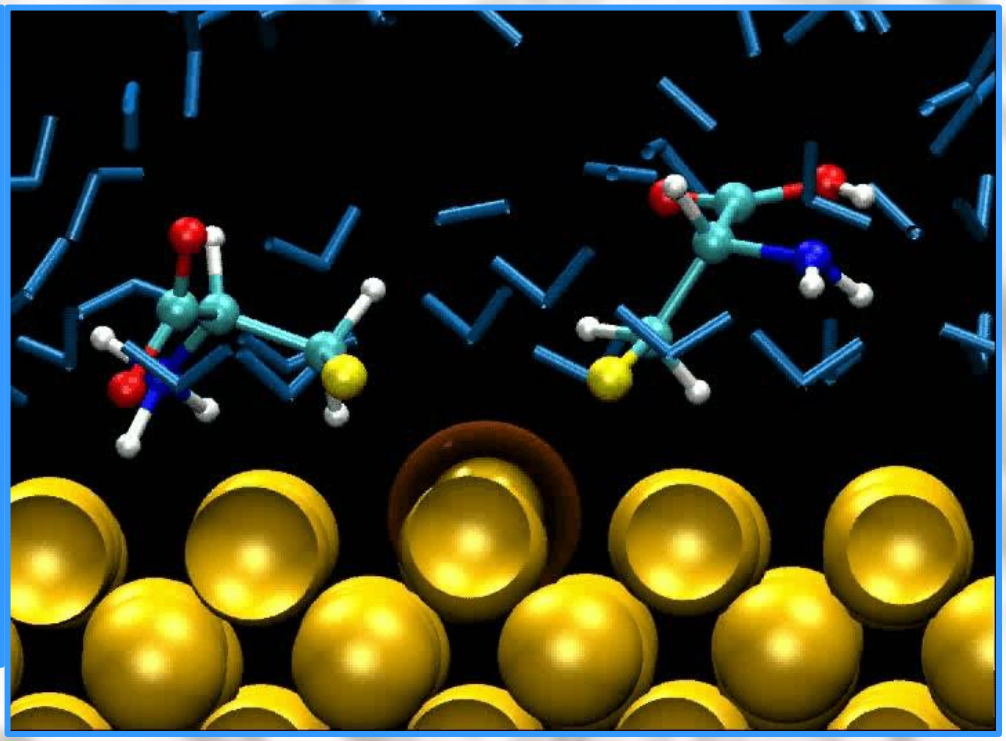


Validation →



← Oxygen & Nitrogen Relocation



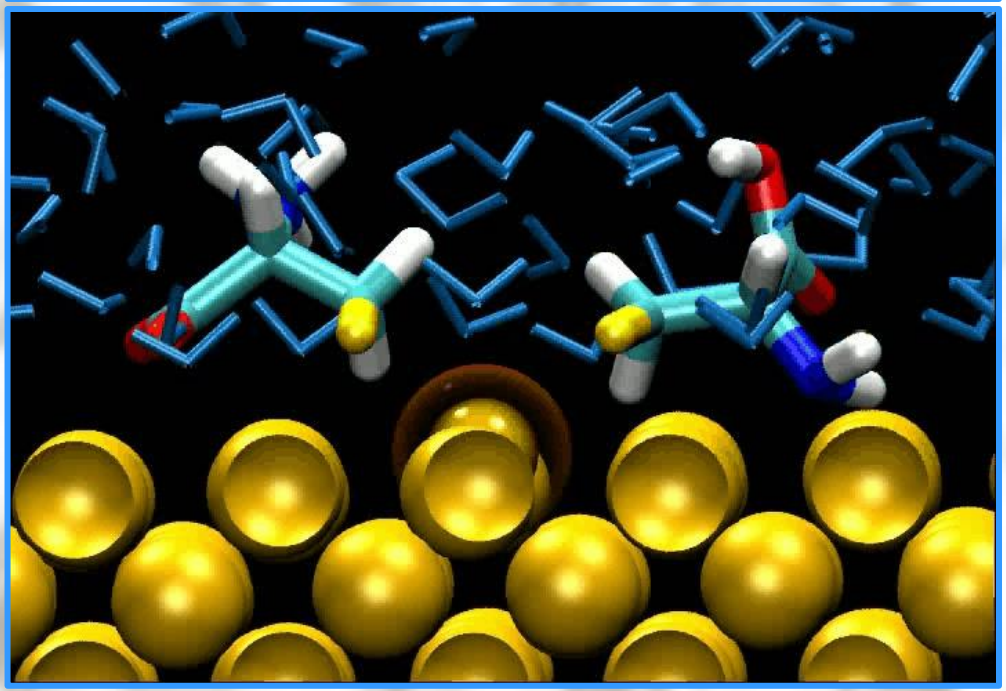


Adsorption of CYSTINE on the Au(110) Surface in Solution

Parallel Orientation

Three stages:

1) Disulfide cleavage
2) Nitrogen Coordination
3) Staple Formation



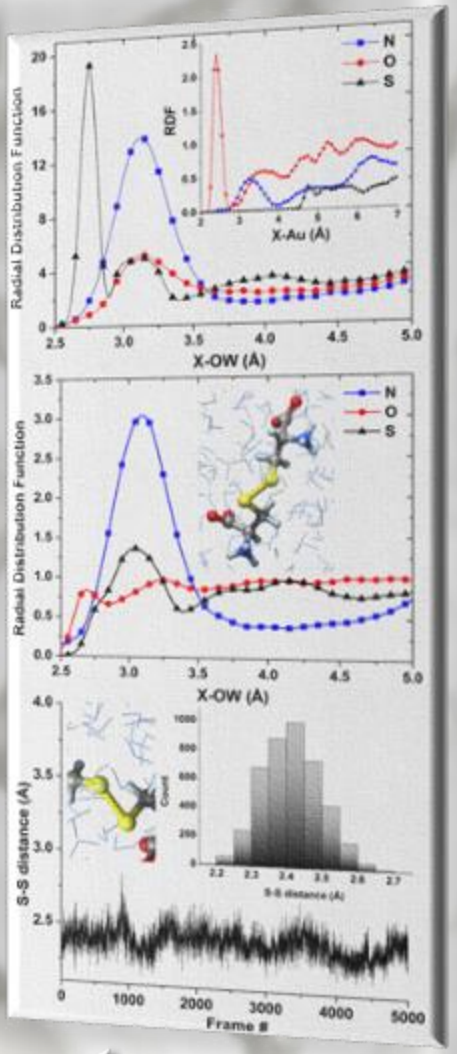
Cystine on Au(110): Single Adsorption

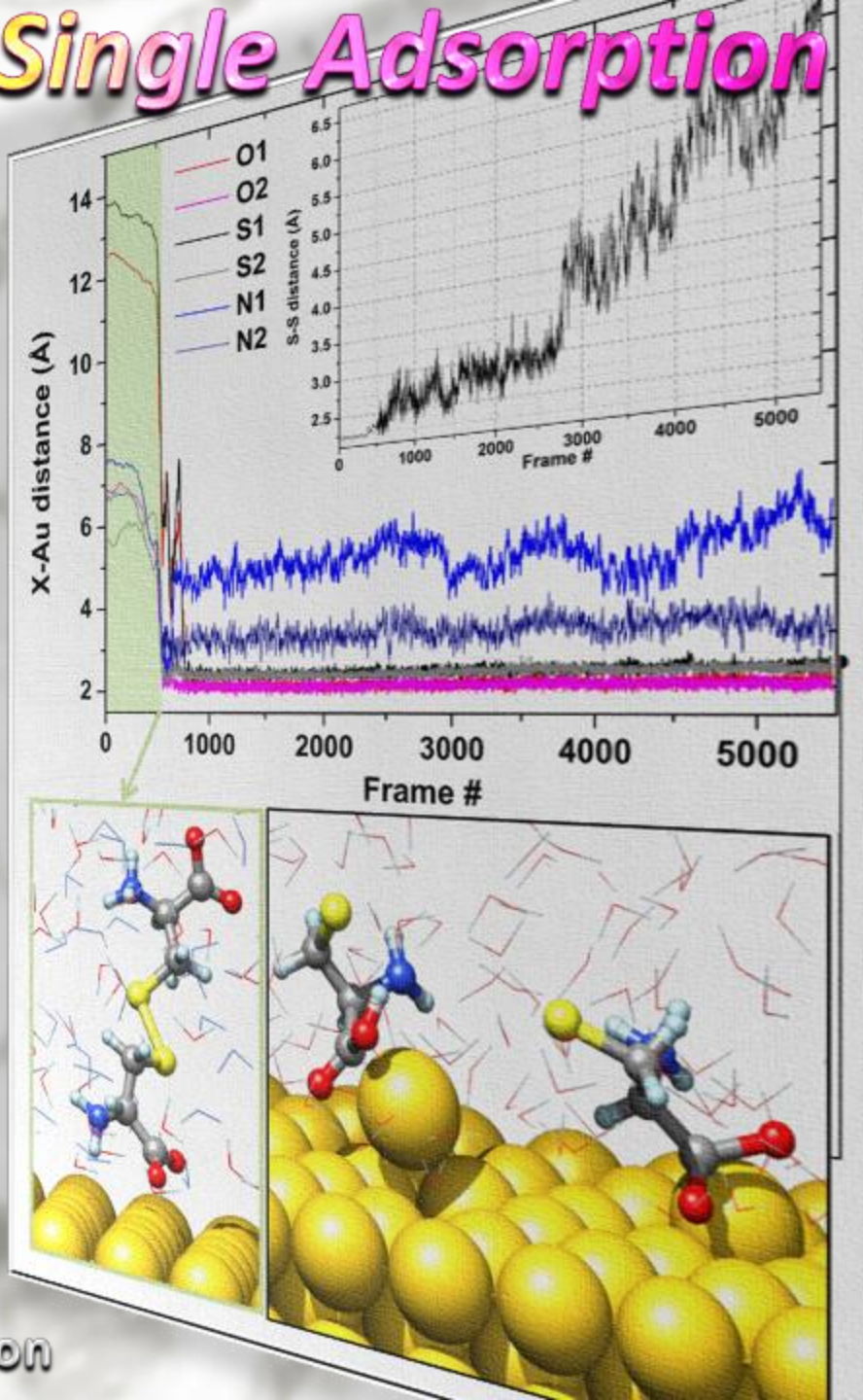
Perpendicular Orientation

Bending of the Molecule towards the surface

Disulfide bond breaking

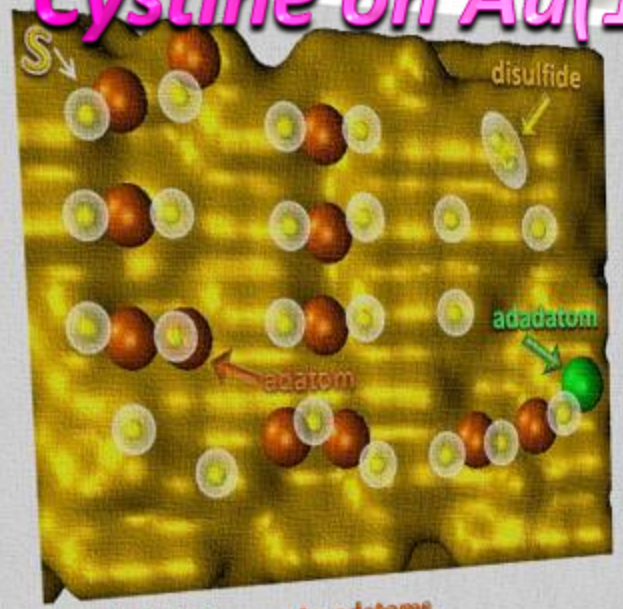
Separate Adsorption of the Monomers

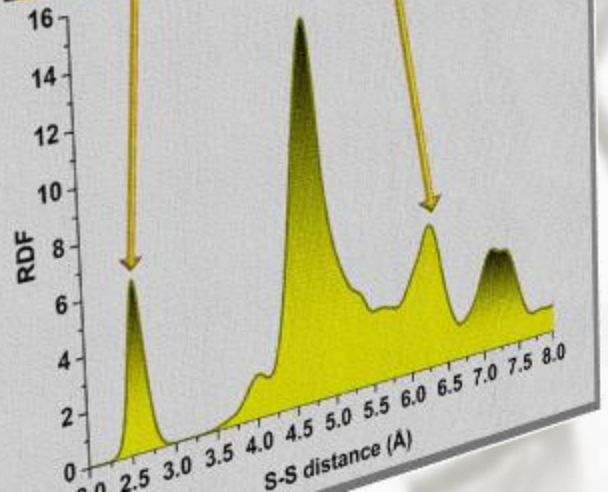


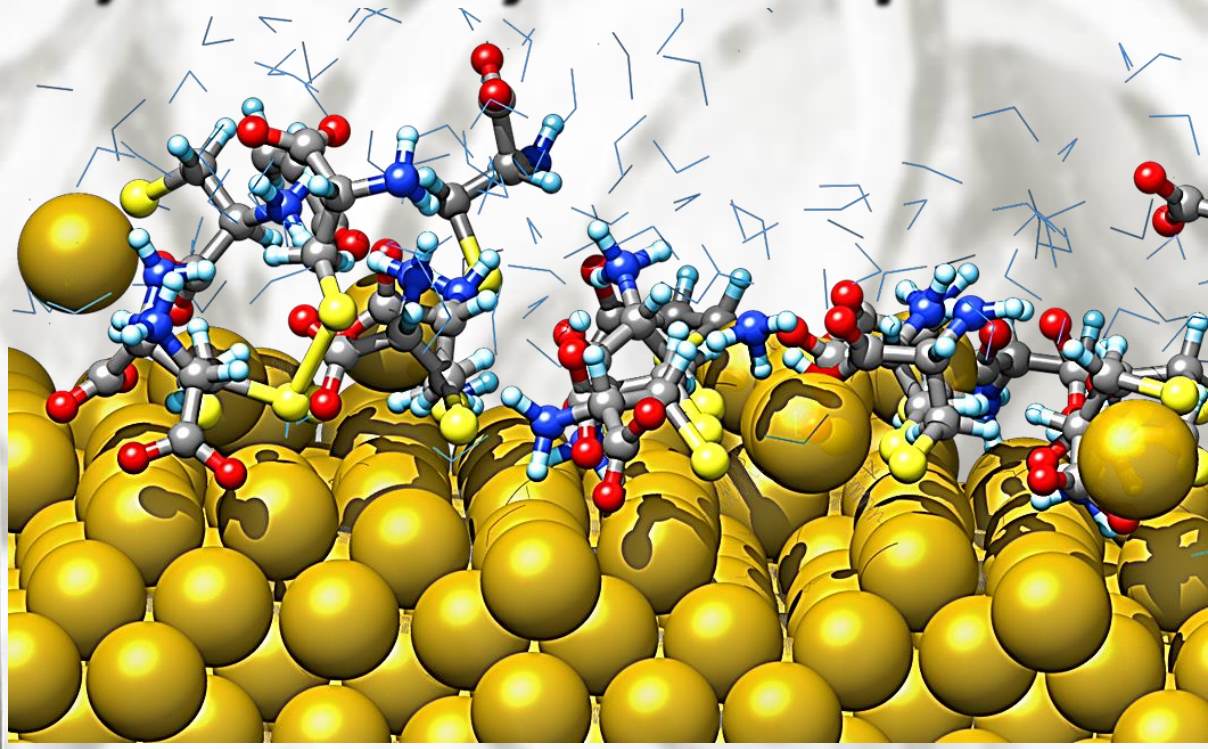


Solvation on the Surface and in Solution

Cystine on Au(110): Multi-layer Adsorption





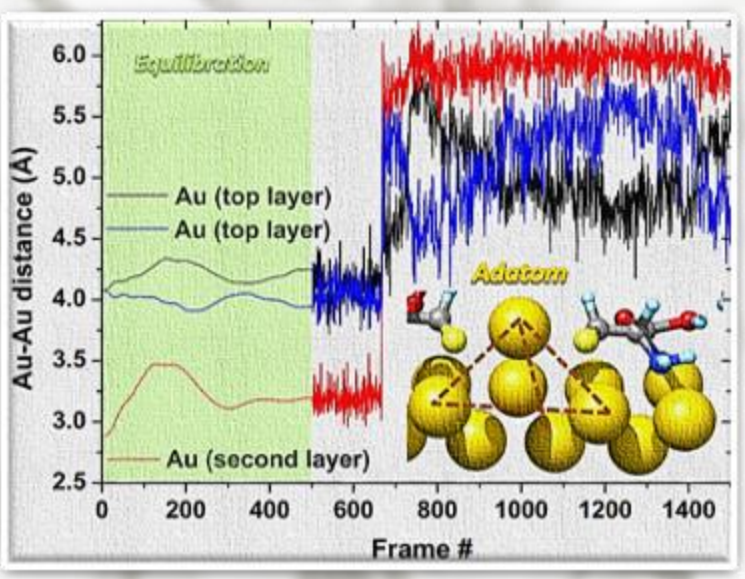


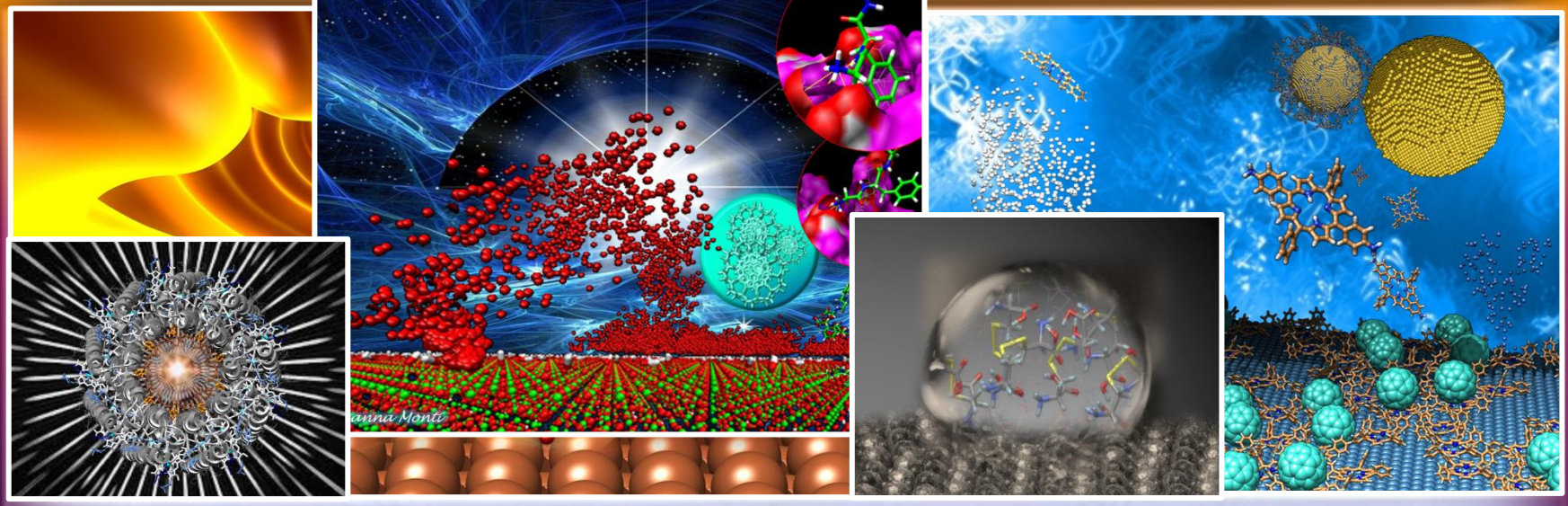
Tweve Cystines in Parallel Orientation

Disulfide Bond Breaking

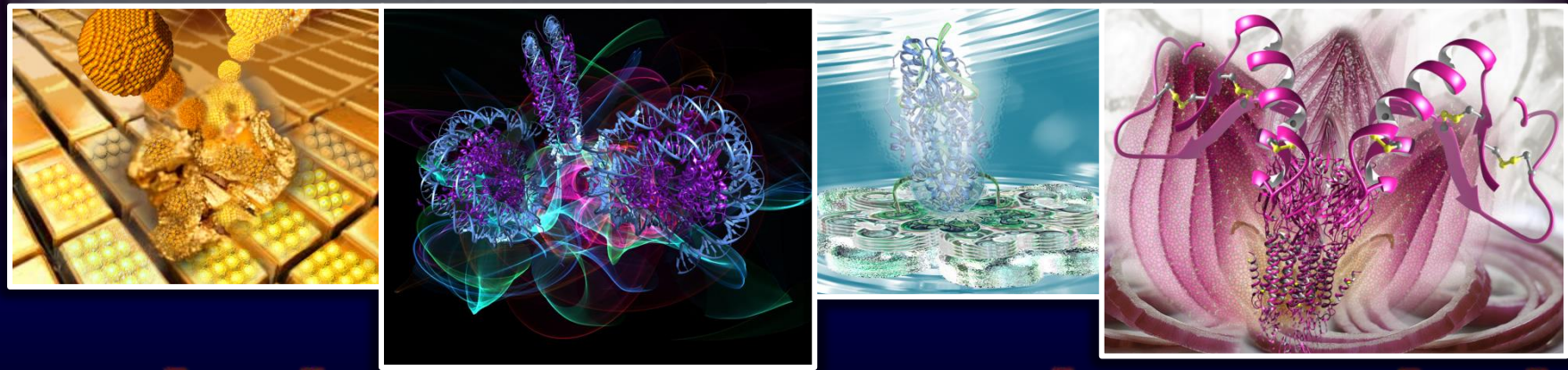
Surface Adatoms

Staples Formation





The NeverEnding Story



In the beginning, it is always *dark*

The Childlike Empress