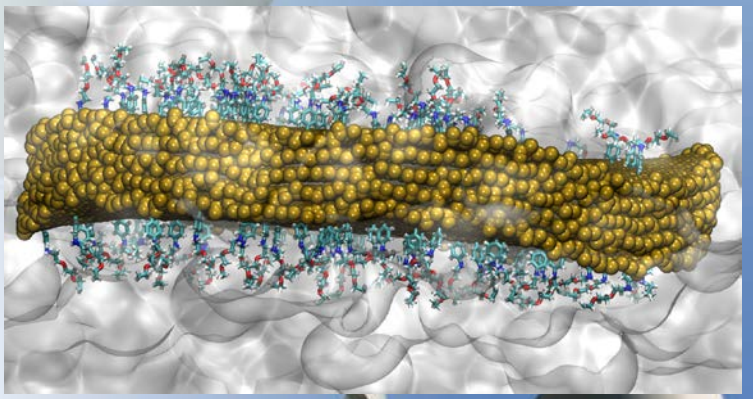
Washington DC, April 2018.

Molecular Modeling of Bio-nano Interfaces for Possibilities in Bio-sensing and Bio-imaging



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CHEMICAL REVIEWS

Review

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Biointerface Structural Effects on the Properties and Applications of Bioinspired Peptide-Based Nanomaterials

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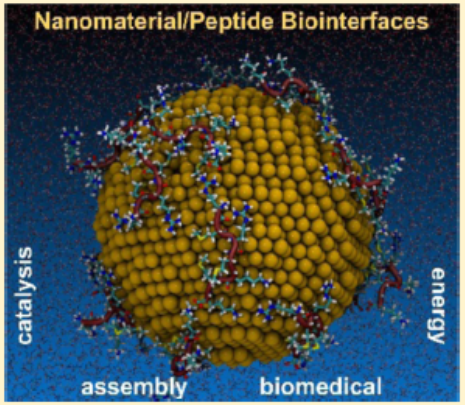
‡Department of Chemistry, University of Miami, 1301 Memorial Drive, Coral Gables, Florida 33146, United States

ABSTRACT: Peptide sequences are known to recognize and bind different nanomaterial surfaces, which has resulted in the screening and identification of hundreds of peptides with the ability to bind to a wide range of metallic, metal oxide, mineral, and polymer substrates. These biomolecules are able to bind to materials with relatively high affinity, resulting in the generation of a complex biointerface between the biotic and abiotic components. While the number of material-binding sequences is large, at present, quantitative materials-binding characterization of these peptides has been accomplished only for a relatively small number of sequences. Moreover, it is currently very challenging to determine the molecular-level structure(s) of these peptides in the materials adsorbed state. Despite this lack of data related to the structure and function of this remarkable biointerface, several of these peptide sequences have found extensive use in creating functional nanostructured materials for assembly, catalysis, energy, and medicine, all of which are dependent on the structure of the individual peptides and collective biointerface at the material surface. In this Review, we provide a comprehensive overview of these applications and illustrate how the versatility of this peptide-mediated approach for the growth, organization, and activation of nanomaterials could be more widely expanded via the elucidation of biointerfacial structure/property relationships. Future directions and grand challenges to realize these goals are highlighted for both experimental characterization and molecular-simulation strategies.



ACCOUNTS of chemical research

Article

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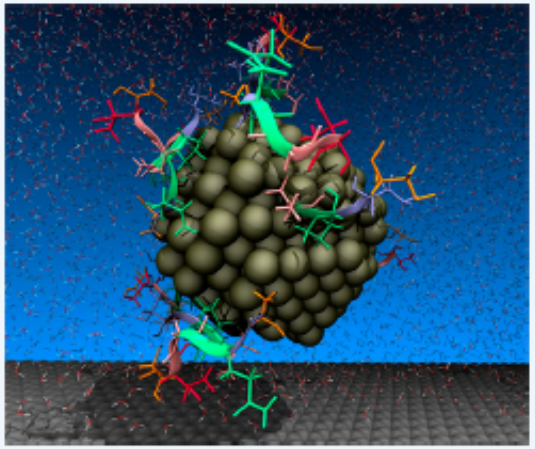
Pathways to Structure–Property Relationships of Peptide–Materials Interfaces: Challenges in Predicting Molecular Structures

Tiffany R. Walsh*

Institute for Frontier Materials, Deakin University, Geelong, VIC 3216, Australia

CONSPECTUS: An in-depth appreciation of how to manipulate the molecular-level recognition between peptides and aqueous materials interfaces, including nanoparticles, will advance technologies based on self-organized metamaterials for photonics and plasmonics, biosensing, catalysis, energy generation and harvesting, and nanomedicine. Exploitation of the materials-selective binding of biomolecules is pivotal to success in these areas and may be particularly key to producing new hierarchically structured biobased materials. These applications could be accomplished by realizing preferential adsorption of a given biomolecule onto one materials composition over another, one surface facet over another, or one crystalline polymorph over another. Deeper knowledge of the aqueous abiotic–biotic interface, to establish clear structure–property relationships in these systems, is needed to meet this goal.



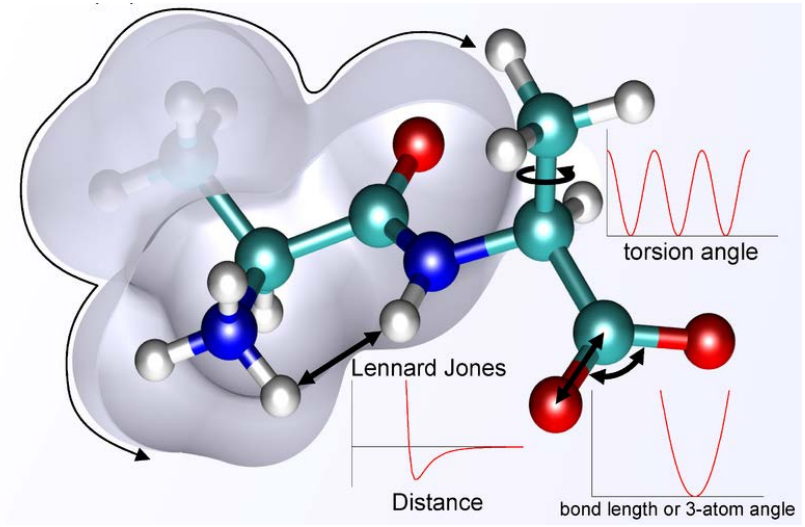
In particular, a thorough structural characterization of the surface-adsorbed peptides is essential for establishing these relationships but can often be challenging to accomplish via experimental approaches alone. In addition to myriad existing challenges associated with determining the detailed molecular structure of any molecule adsorbed at an aqueous interface, experimental characterization of materials-binding peptides brings new, complex challenges because many materials-binding peptides are thought to be intrinsically disordered. This means that these peptides are not amenable to experimental techniques that rely on the presence of well-defined secondary structure in the peptide when in the adsorbed state. To address this challenge, and in partnership with experiment, molecular simulations at the atomistic level can bring complementary and critical insights into the origins of this abiotic/biotic recognition and suggest routes for manipulating this phenomenon to realize new types of hybrid materials.

For the reasons outlined above, molecular simulation approaches also face challenges in their successful application to model the biotic–abiotic interface, related to several factors. For instance, simulations require a plausible description of the chemistry and

Molecular dynamics simulations: the basics

- We need to describe the interactions between *all* the atoms in the system.

Can simulate millions of atoms using this approach (with supercomputing facilities).



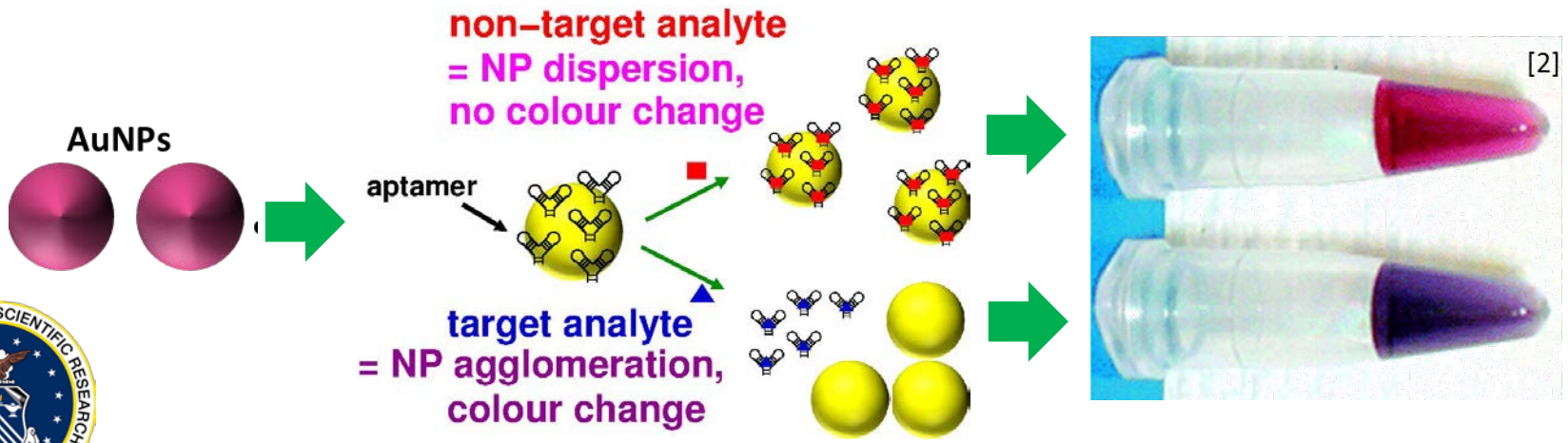
MD simulations of bio-interfaces:
very challenging, requires vast computing resources.

Access to supercomputing is critical for our research.

Biomolecule-materials interfaces: Possibilities for Bio-sensing

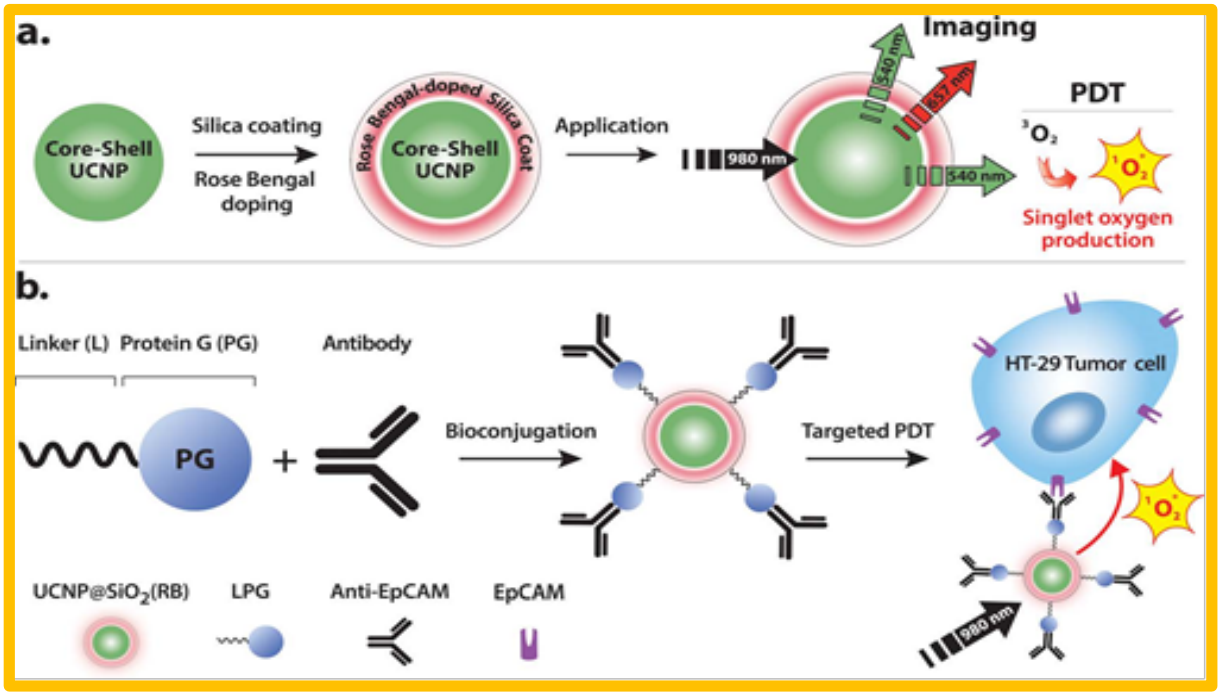
The goal: *in-situ* real-time monitoring of molecular biomarkers for vigilance, stress and fatigue.

Non-covalent adsorption of Au nanoparticles by nucleic acid aptamers (**DNA** *etc*) that bind biomarkers, *e.g.* cortisol.
→ colorimetric sensor.



[2] Rothberg et al., *J. Am. Chem. Soc.*, 2004, **126**, 10958.

Biomolecule-materials interfaces: Possibilities for Bio-imaging



Khaydukovet al.. *Sci. Rep.* **2016,** *6*, 35103

Peptide coatings on nanoparticles can ***target*** specific sites for imaging, and also for delivery of therapeutics.

Centre for Nanoscale BioPhotonics
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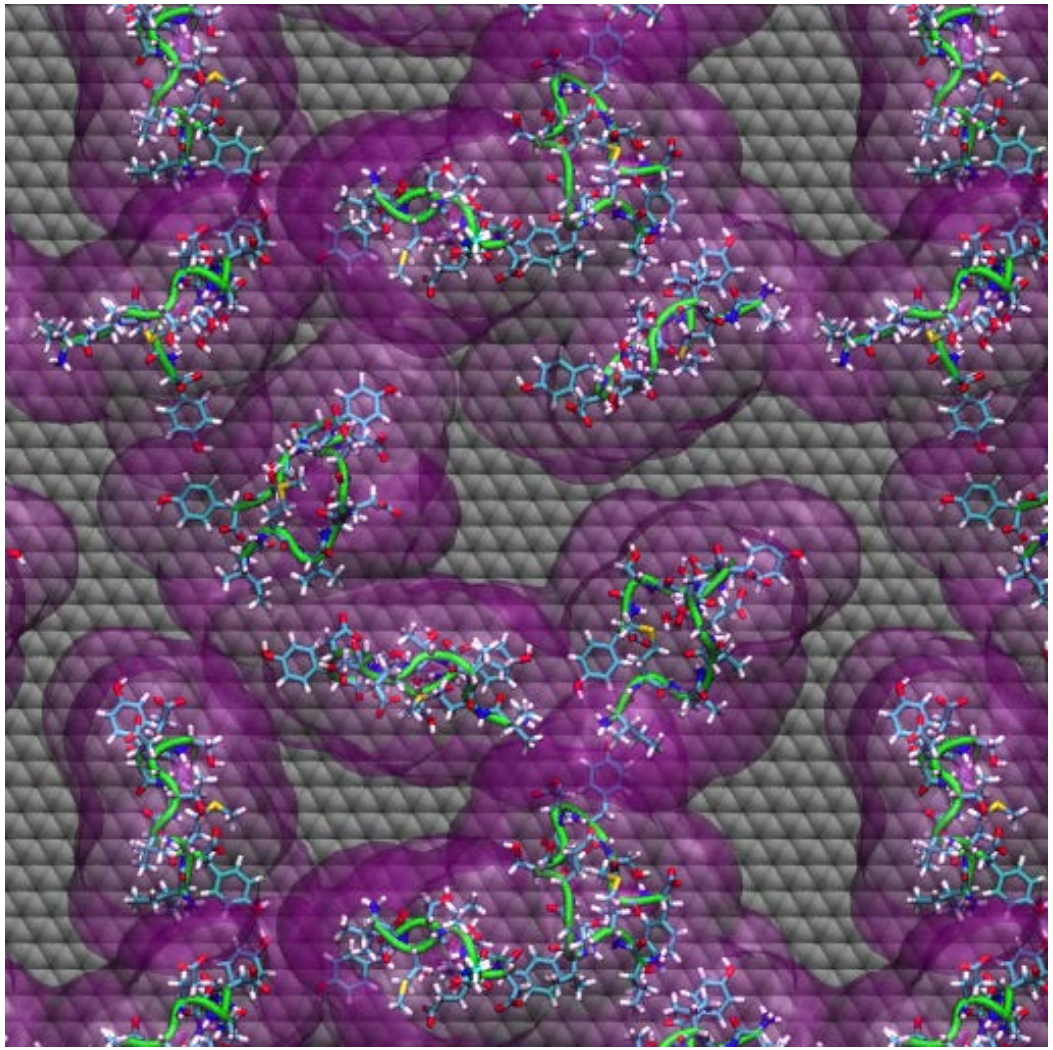
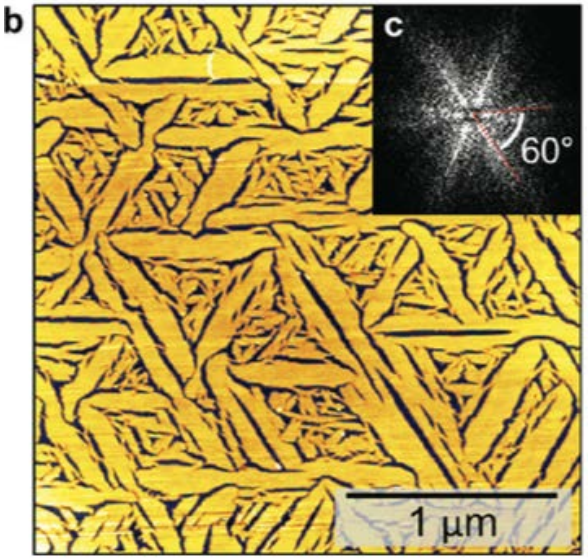
Molecular modelling can help our understanding of how to achieve imaging at new limits of **resolution**, **sensitivity** and **specificity**.

Peptide Self-Organization and Surface Patterning





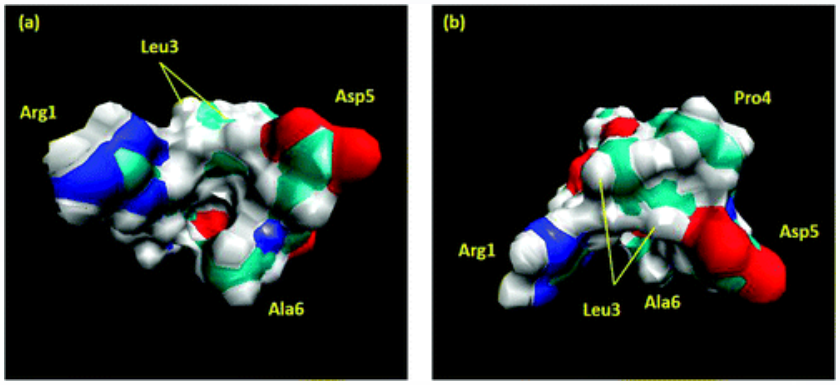
Simulations performed at 50% and 70% peptide surface coverage

Patterns were measured in using ***ex-situ* AFM**. We predict these do not persist in water.

Hughes and Walsh, Nanoscale, 2018

Nexus of Sequence/Structure(s)/Binding in Bio-interfaces:

sequence ↔ 3D structures ↔ binding ↔ properties



Mirau, Naik Gehring, JACS (2011)

Experimental structural determination of the aqueous bio-interface is challenging.

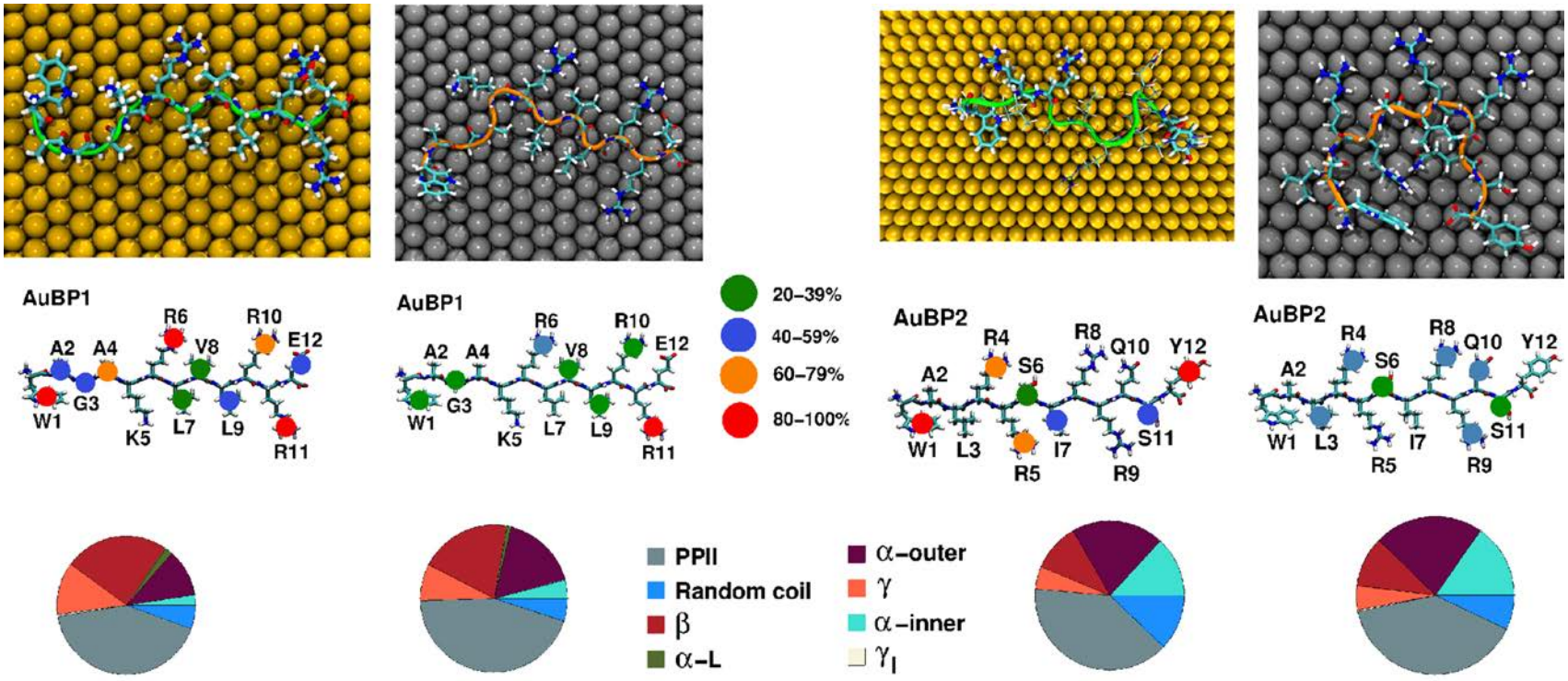
Molecular simulation can help us to unplait the threads of this nexus.

Peptide sequence matters for materials-selective binding:

✓ ✓ **AQNPSDNNTHTH** *vs.* **TNHDHSNAPTNQ** ✗ ✗

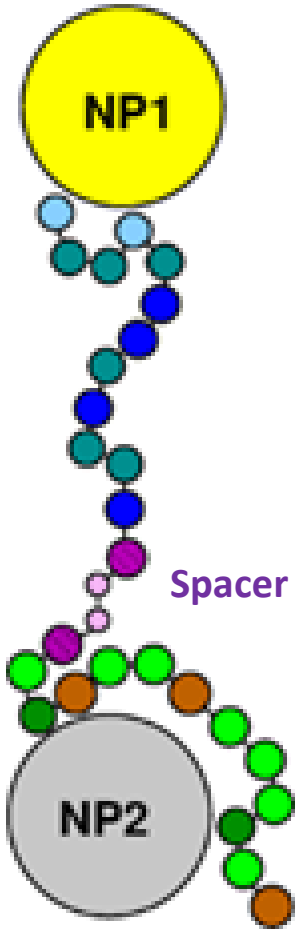
Compositionally-selective peptide binding: Ag and Au surfaces

Peptide adsorption selectivity of gold over silver surfaces:
We used MD simulations to explore experimental binding affinity vs. our structural predictions.



Palafox-Hernandex, Tang, Hughes, Li, Siwhart, Prasad, Walsh and Knecht , Chem. Mater. (2014)

Adding a photoswitch to a materials–binding peptide



Materials-binding domain 1

Spacer

Materials-binding domain 2

Concept:

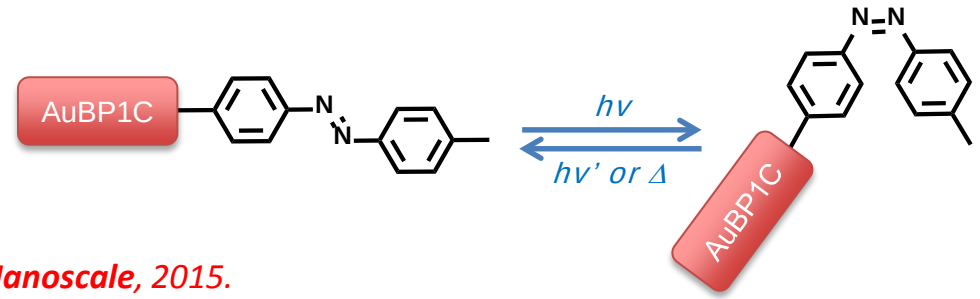
Attach a photo-active molecular spacer to a chimeric peptide.

Here, the Spacer located between the two domains could be reversibly switched between stable conformations, to create photo-activated, reconfigurable bio/nano interfaces.

But….this modification may disrupt the materials-selective properties of the peptide.

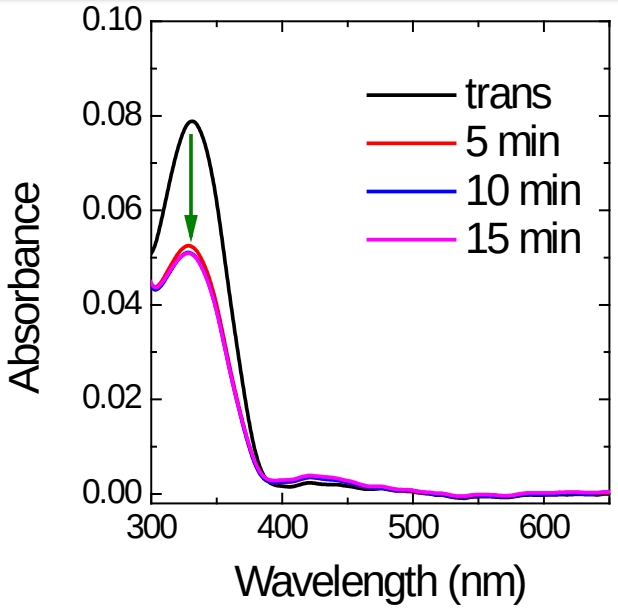
Knecht, Walsh, Swihart, Prasad and co-workers.

Photoisomerization of Free AuBP1-MAM

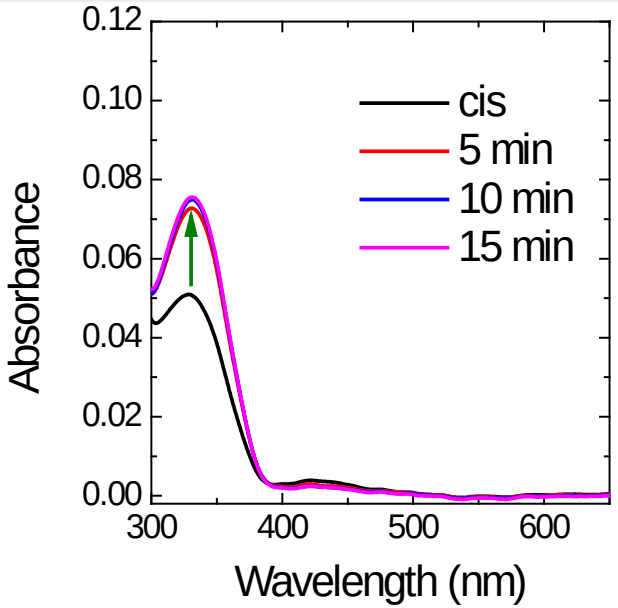


Tang et al., Walsh & Knecht, ***Nanoscale****, 2015.*

trans to *cis*
under 365 nm irradiation

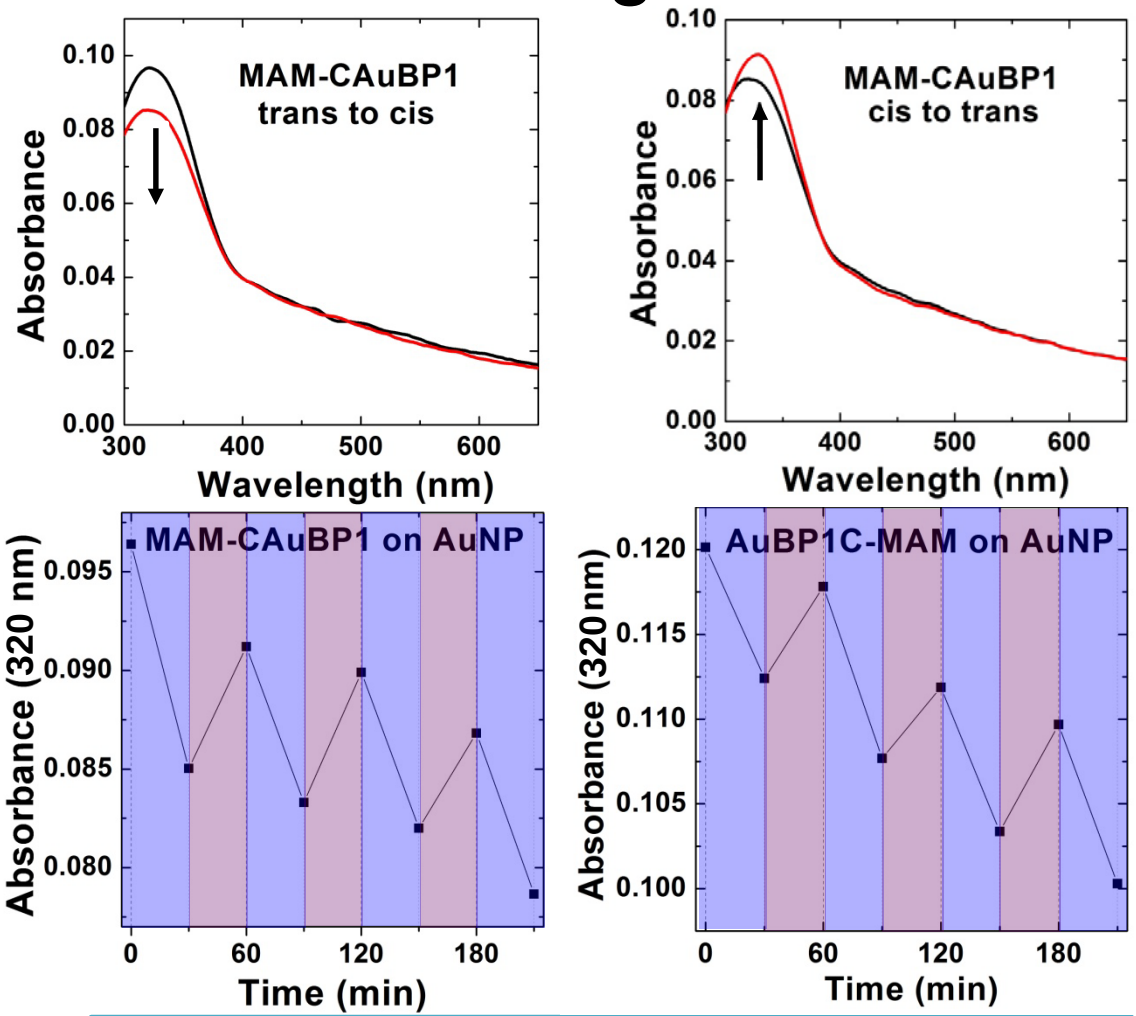


cis to *trans*
under 440 nm irradiation



Conjugation to peptide did not inhibit photoswitching of the azo group

Photoswitching on Au NPs



Surface bound AuBP1-MAM on Au NPs can be reversibly switched many times. But the switching ratio is lesser than free AuBP1-MAM.

Tang, Lim, Palafox-Hernandez, Drew, Li, Swihart, Prasad, Walsh, Knecht, *Nanoscale, 2015.*

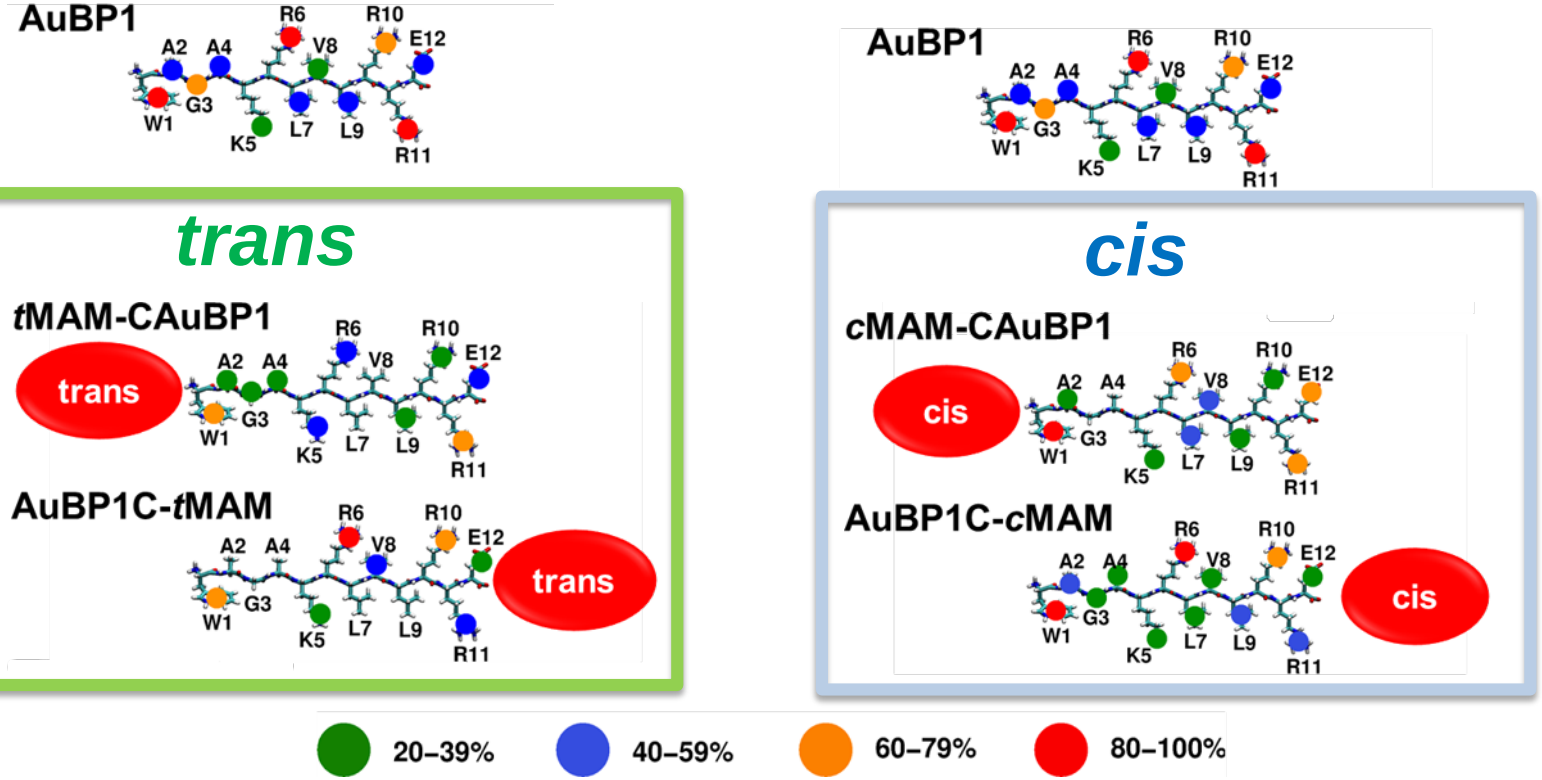
QCM Binding Analysis: Comparison of Au to Ag

Au and Ag binding affinities of hybrid peptide-MAM structures are comparable to parent peptide, in both *cis* and *trans* forms

Molecule	Material	ΔG (kJ/mol)	Θ_∞ (%)
AuBP1	Au Ag	−37.6 ± 0.9 −35.3 ± 0.8	97.6 94.2
*t*MAM-CAuBP1	Au Ag	−37.1 ± 1.7 −35.8 ± 0.7	96.0 89.5
*c*MAM-CAuBP1	Au Ag	−34.3 ± 0.7 −35.7 ± 1.0	88.5 90.2
AuBP1C-*t*MAM	Au Ag	−34.8 ± 1.1 −35.6 ± 0.6	90.3 89.6
AuBP1C-*c*MAM	Au Ag	−35.5 ± 0.0 −34.6 ± 0.6	92.6 85.3

Palafox-Hernandez, et al.,Knecht and Walsh, ***ACS Appl. Mater. Interfaces***, *2016.*

Surface-Residue Contact:
Predictions at aqueous Au interface

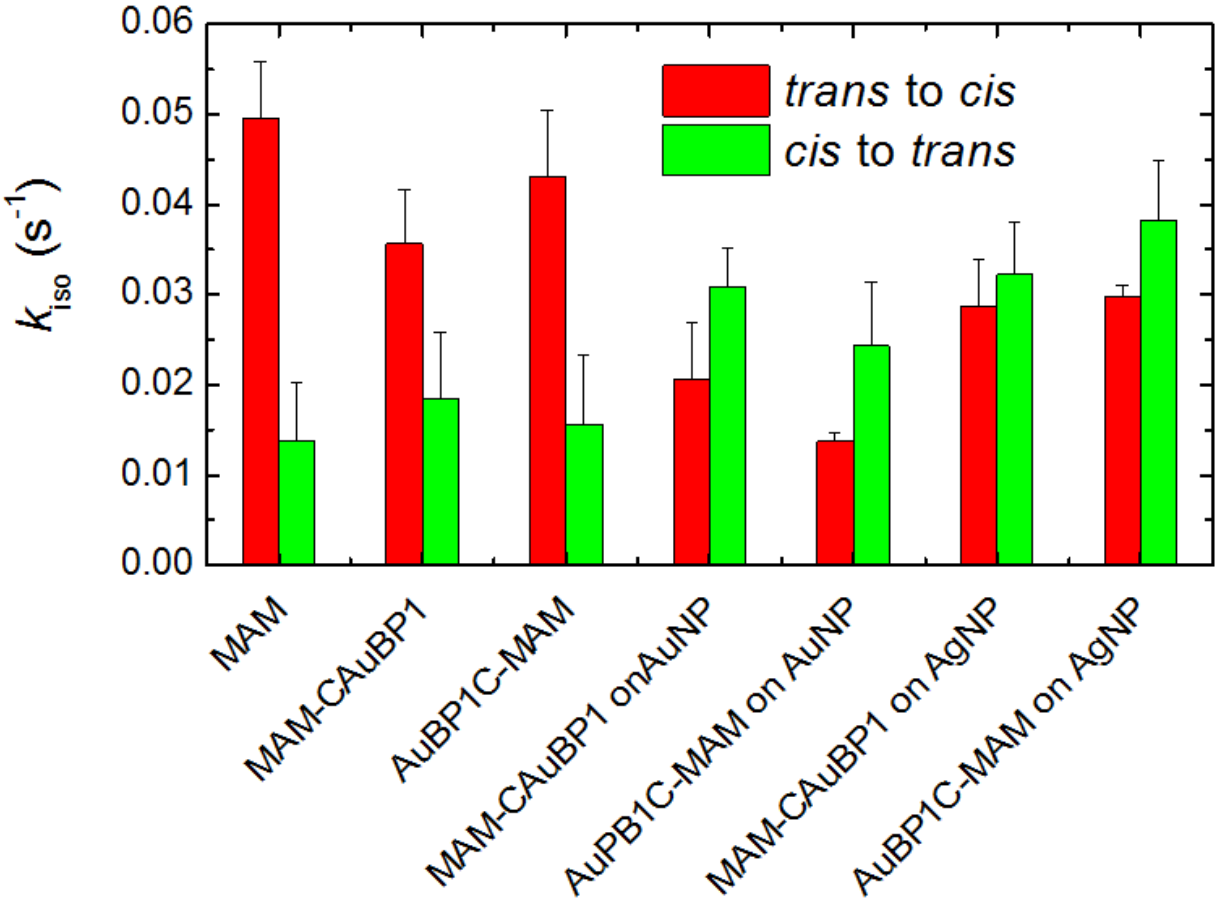


The location and isomerization state of the MAM affect the peptide/Au binding, but AuBP1 remains enthalpically-driven, with strong anchor sites, for both *cis* and *trans*.

Palafox-Hernandez, et al.,Knecht and Walsh, ***ACS Appl. Mater. Interfaces***, *2016.*

Photoswitching Rate Constant

Free molecules – *trans* to *cis* switching is faster.
Adsorbed on NPs – *cis* to *trans* switching is faster.



*Calculated binding free energy (kJ mol^{-1})

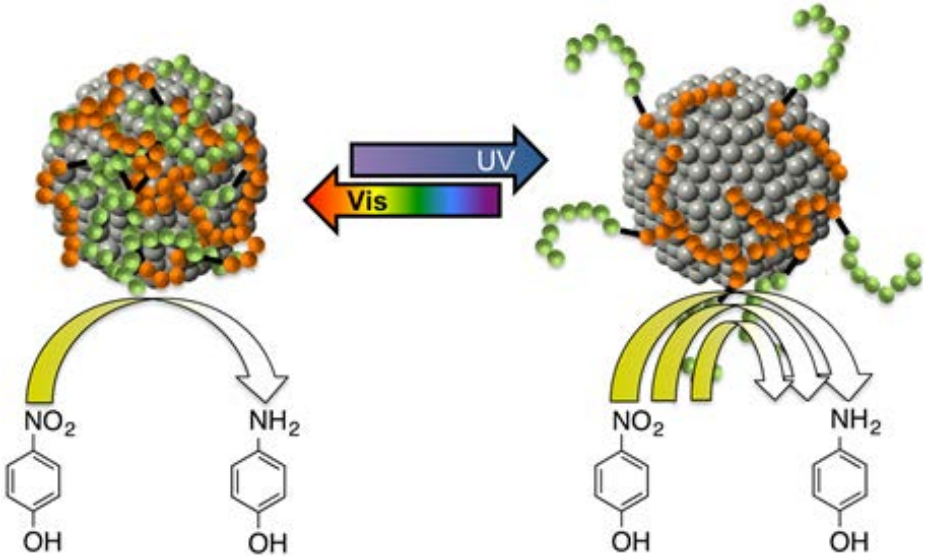
Metal	trans-MAM	cis-MAM
Au	−102.9 ± 3.9	−62.9 ± 8.2
Ag	−45.5 ± 4.7	−29.5 ± 5.5

Strong interaction between metal and trans-MAM inhibits trans to cis isomerization and accelerates cis to trans isomerization.

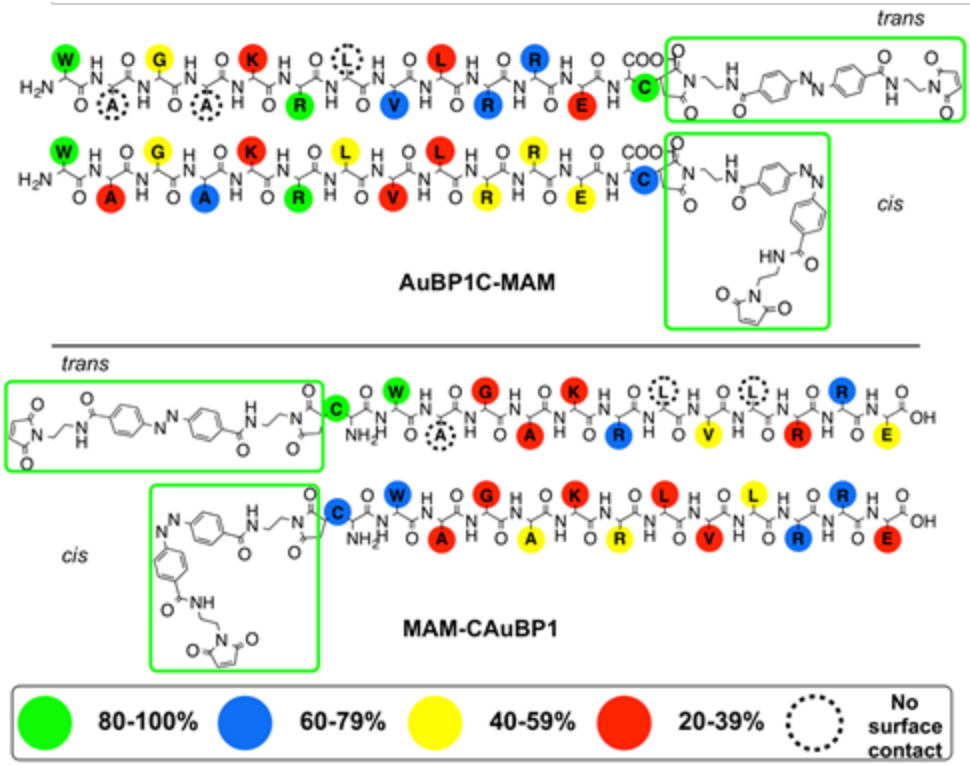
Switching behaviors can be directly modulated by the metallic interface due to the specific interactions between the metal and the azobenzene.

Palafox-Hernandez, et al.,Knecht and Walsh, ***ACS Appl. Mater. Interfaces***, *2016.*

Photoswitchable Catalytic Activity



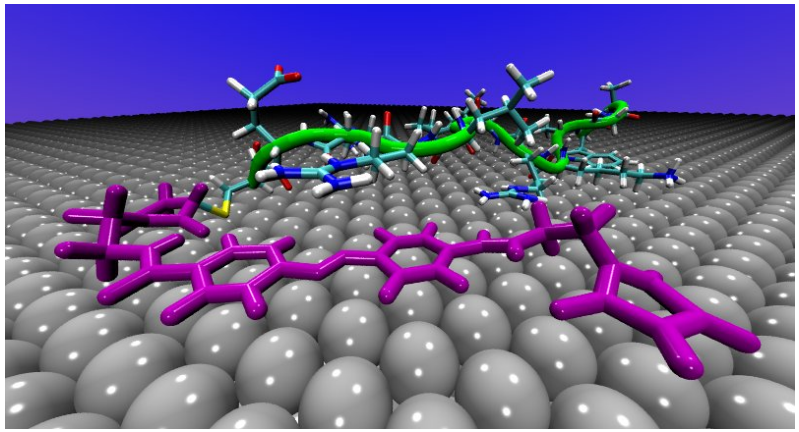
Photoswitching of azobenzene moiety modifies surface accessibility, producing in a change in catalytic activity



Contact pattern of materials-binding peptide changes upon switching of MAM component

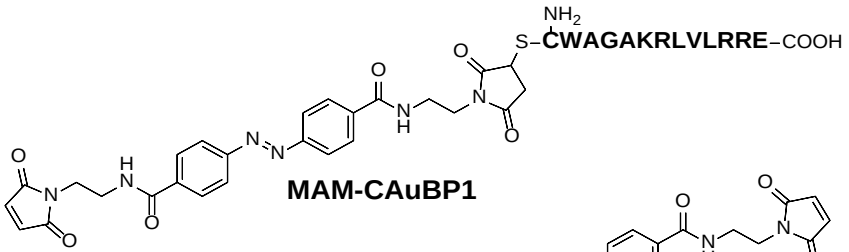
Lawrence, Scola, Li, Lim, Liu, Prasad, Swihart, and Knecht, *ACS Nano*, **2016**, 9470–9477.

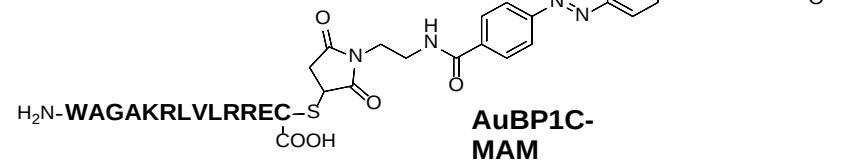
Applications to Non-Linear Optics

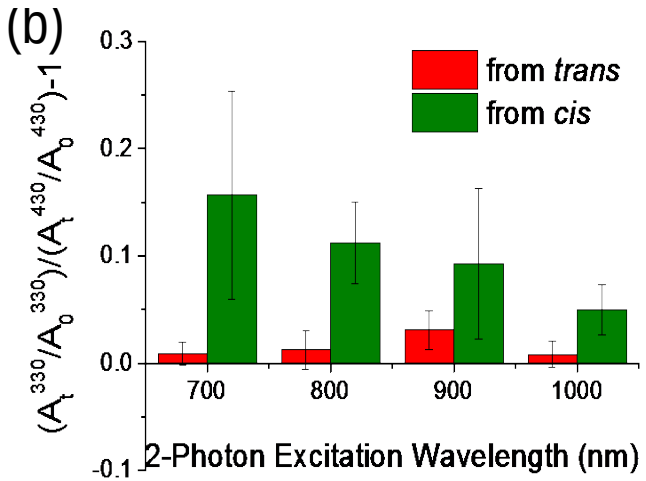


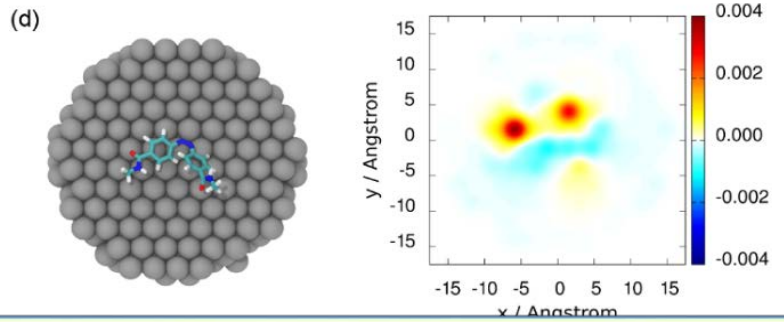
H_2N–**CWAGAKRLVLRRE**-COOH
CAuBP1

H_2N-**WAGAKRLVLRREC**-COOH
AuBP1C





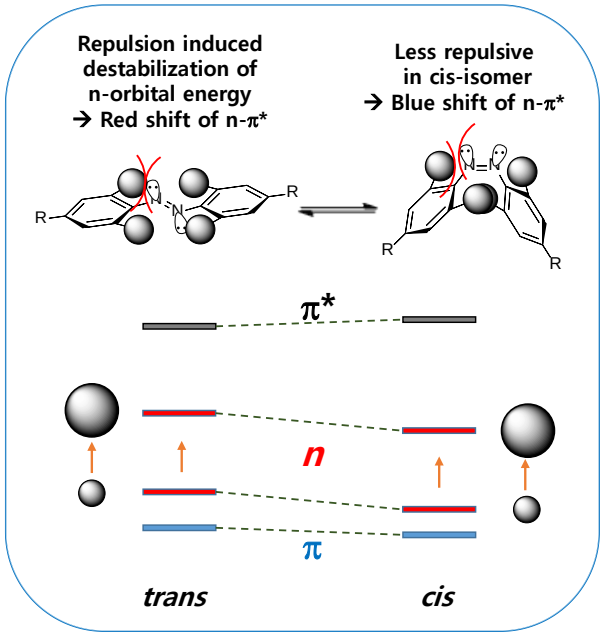




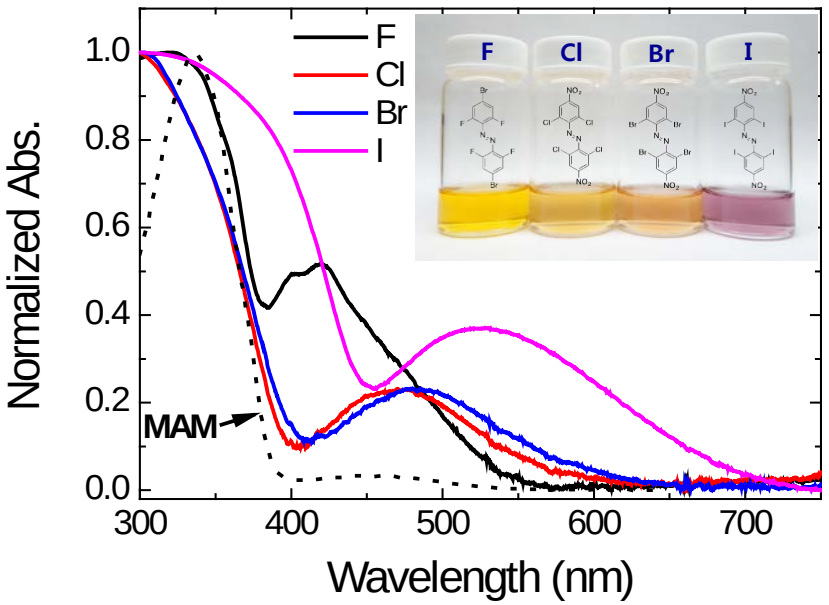
LSPR-assisted two-photon induced isomerization for peptide-azobenzene by non-covalent coupling with Ag nanoparticles. Greatest effect for ***cis*** state.

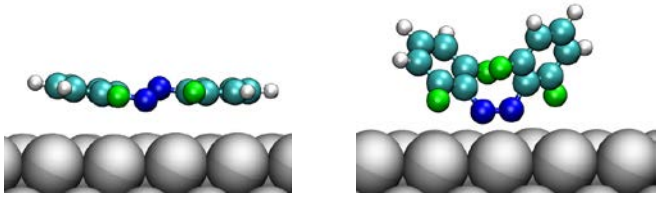
Ågren, Prasad, Swihart, Knecht , Walsh, and co-workers, ***Nanoscale****, 2016.*

o-Haloazobenzene Substitution to Tune Photoswitching Wavelengths



Abs. spectra of MAM and *o*-Haloazobenzenes





	MAM	F	Cl	Br	I
n-π^* λ_{max} (nm)	450	420	470	485	525

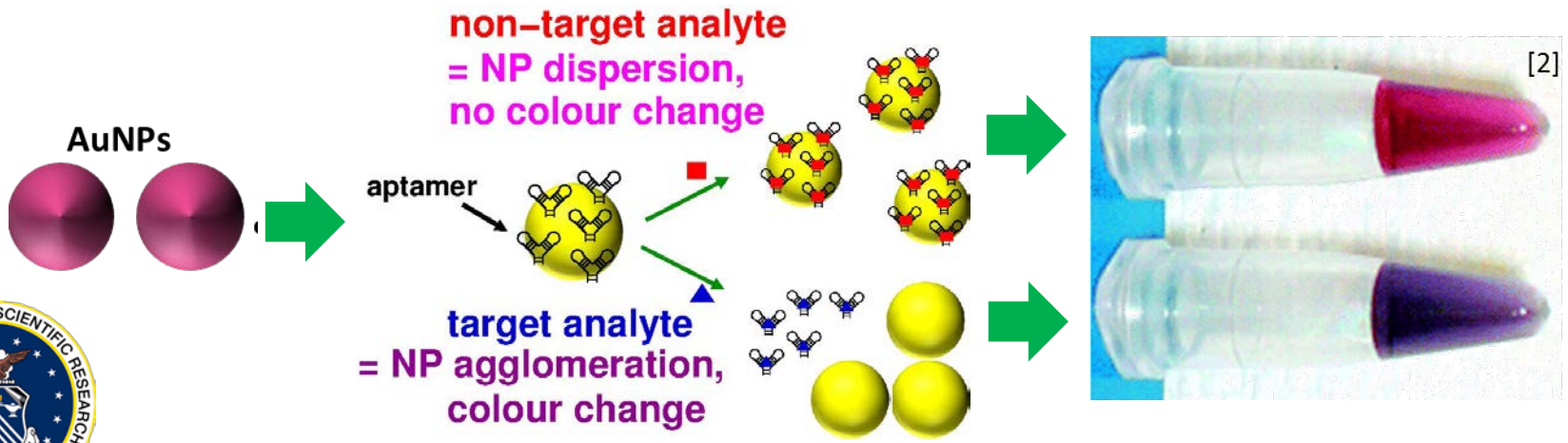
o-halogen *substitution can tune the photoswitching wavelengths; competing effects can result in net blue-shift (F) or red-shift (Cl, Br, or I)*

Hughes, Baev, Prasad and Walsh, ***Phys. Rev. B***, *2017*

Biomolecule-materials interfaces: Possibilities for Bio-sensing

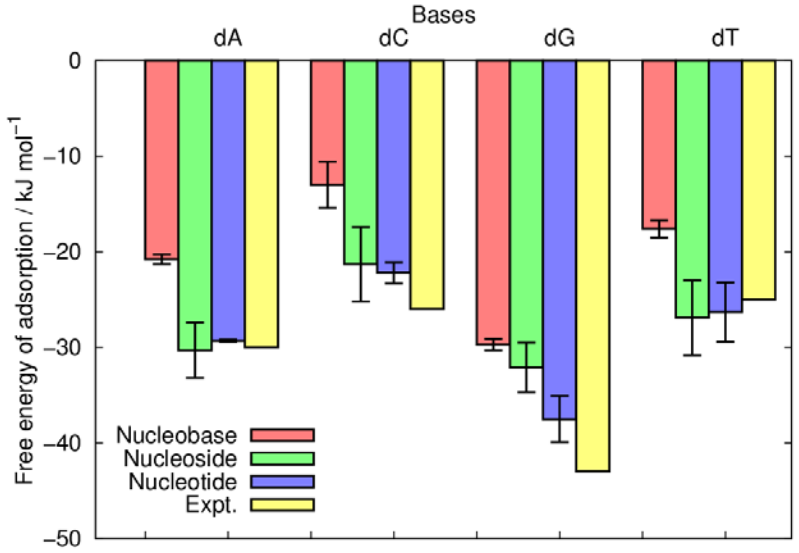
The goal: *in-situ* real-time monitoring of molecular biomarkers for vigilance, stress and fatigue.

Non-covalent adsorption of Au nanoparticles by nucleic acid aptamers (**DNA** *etc*) that bind biomarkers, *e.g.* cortisol.
→ colorimetric sensor.

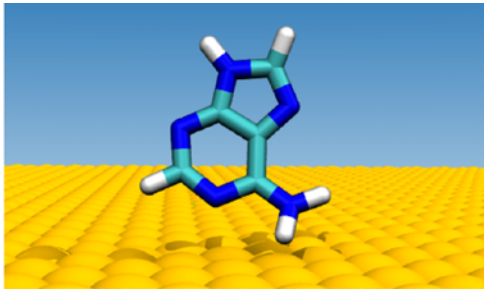


[2] Rothberg et al., *J. Am. Chem. Soc.*, 2004, **126**, 10958.

Digression: Modeling Interactions Between DNA and Au surfaces – How to Validate?



We have adapted and verified force-fields for describing the interface between nucleic acids and gold (and graphene).



We used metadynamics simulations to predict the binding free energy of nucleic acid components at the aqueous Au & graphene interfaces, consistent with Single Molecule Force Spectroscopy AFM experimental data.

Hughes, Drew, Wei, Colombi Ciacchi, and Walsh, Langmuir, 2017.

Digression: Modeling Interactions Between DNA and Au surfaces – How to Validate?

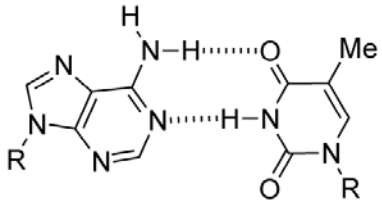
Table 3. Summary of Estimated Free Energies of Adsorption, ΔFE_{ads}, of DNA Fragments to Aqueous Graphitic and Gold Interfaces, Including Estimates from Previous Work Both Experimental and Simulation

				ΔFE_{ads} (kJ mol^{-1})			
substrate	reference	method	adsorbate[a]	dA	dC	dG	dT
graphitic	present work	SMFS	O	−38 ± 5	−32 ± 3	−53 ± 11	−33 ± 8
	Iliafar[34,35]	SMFS	O	−41.4 ± 2.1	−31.4 ± 3.3	−34.7 ± 0.8	−47.3 ± 3.3
	Ranganathan[43b]	ITC	NS	−26.0 ± 0.2	−22.8 ± 0.2	−26.4 ± 0.5	−21.6 ± 1.5
	present work	Sim	NB	−22.5 ± 1.0	−12.9 ± 0.9	−27.7 ± 1.6	−16.5 ± 1.0
	present work	Sim	NS	−25.2 ± 2.1	−18.0 ± 2.5	−35.4 ± 1.9	−25.6 ± 1.3
	present work	Sim	NT	−27.1 ± 2.5	−21.4 ± 1.0	−38.3 ± 2.0	−27.9 ± 2.1
	Johnson[46c]	Sim	NB	−35.1 ± 1.3	−26.8 ± 1.7	−43.1 ± 1.7	−32.2 ± 1.7
	Ranganathan[43d]	Sim	NS	−58	−46	−67	−52
	Ranganathan[43e]	Sim	NS	−38	−29	−42	−29
	Ranganathan[43f]	Sim	NS	−24	−20	−26	−20
	Shi[47]	Sim	O	−113.0	−100.4	−125.5	−108.8
gold	present work	SMFS	O	−36 ± 11	−21 ± 7	−29 ± 3	−28 ± 2
	Bano[42g]	SMFS	O	−23.6 ± 0.1	−13.3 ± 0.1	−13 ± 0.1	−7.8 ± 0.1
	present work	Sim	NB	−35.4 ± 1.8	−18.5 ± 1.0	−34.6 ± 1.1	−18.3 ± 0.5
	present work	Sim	NS	−39.4 ± 1.8	−26.8 ± 1.7	−38.1 ± 0.9	−21.8 ± 0.9
	present work	Sim	NT	−45.7 ± 3.1	−34.9 ± 1.3	−44.4 ± 1.3	−28.6 ± 1.5

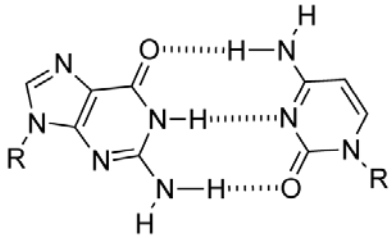
[a]Nucleobase (NB), nucleoside (NS), nucleotide (NT), or oligomer (O). [b]On reduced GO. [c]On (11,0) CNT, AMBER99 parameters. [d]On graphene, AMBER99 parameters. [e]On graphene, Chen−Garcia parameters. [f]On graphene, revised parameters. [g]On gold-coated silicon wafers.

Hughes, Drew, Wei, Colombi Ciacchi, and Walsh, Langmuir, 2017.

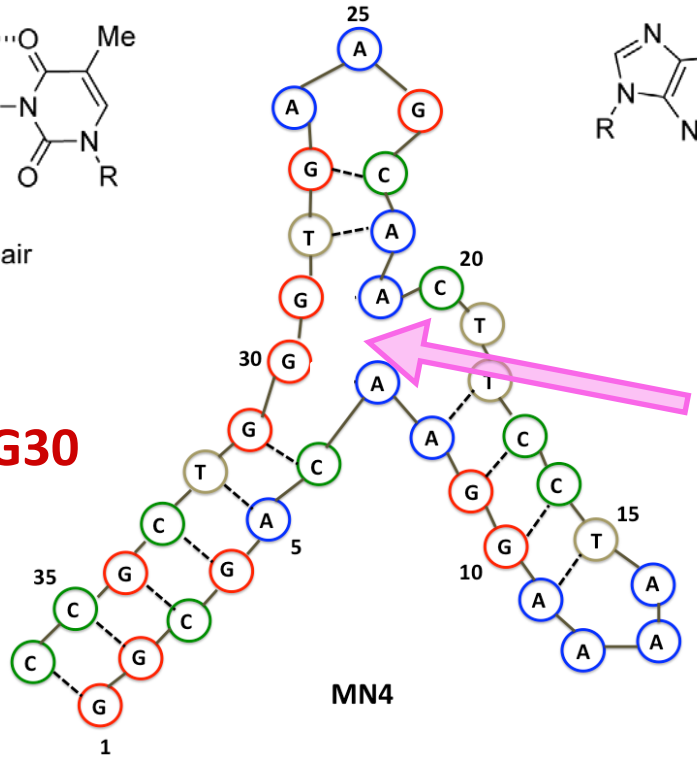
The Cocaine-Binding MN4 Aptamer



A·T base pair



G·C base pair



Junction Bases:

A7, T19, C20, A21, G29, G30

Proposed cocaine-binding site

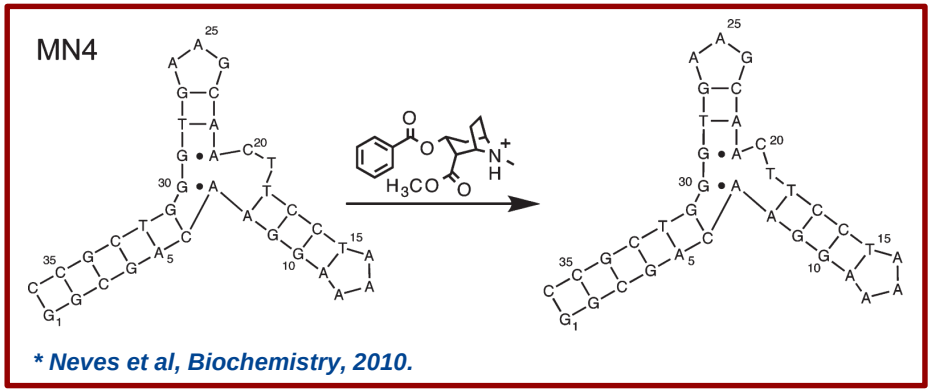
Aptamer Structure: MN4

AFRL researchers have investigated the binding of cocaine to the MN4 aptamer in the presence and absence of Au nanoparticles.

Experimental evidence:

Structure of MN4 = stable in the apo and holo forms*.

Evidence of conformational switching upon binding of cocaine???



** Neves et al, Biochemistry, 2010.*

Challenge:

Currently, there is no reported 3D atomistic-scale structure (pdb) of the MN4 aptamer, in the absence or presence of cocaine.

We need to test if we can predict these structural traits.

WORKFLOW

Summary & Outlook:

- The key deliverable from our modelling is the ability to establish a missing link – **detailed structural data** – between peptide/DNA sequences and the properties of the bio-enabled materials.

sequence ↔ 3D structures ↔ binding ↔ properties

- **Applications for bio-sensing and targeted bio-imaging applications** are the next steps for our integrated program of molecular simulations and experimental characterization.