Dynamic nanoproteins: self-assembled peptide surfaces on monolayer protected gold nanoparticles†

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Here, we demonstrate the formation of dynamic peptide surfaces through the self-assembly of small peptides on the surface of monolayer protected gold nanoparticles. The complexity of the peptide surface can be simply tuned by changing the chemical nature of the added peptides and the ratio in which these are added. The dynamic nature of the surface permits adaptation to changes in the environment.

The exquisite properties of proteins in terms of molecular recognition and catalysis derive in large part from their size and structural complexity.1 Their size permits the presence of internal cavities, which are well-shielded from the bulk solvent2 and permit additional advantages like allosteric control by secondary binding sites3 and multivalent interactions to drive protein–protein interactions.4 The fact that all proteins are composed of a very limited number of amino acids renders these structures even more fascinating. Currently, a strong impetus exists to develop synthetic structures able to match the size and complexity of proteins for applications in diagnostics, medicine and materials science.5,6 The advantage of synthetic structures is the ability to design the desired structure and permit additional advantages like allosteric control by secondary binding sites3 and multivalent interactions to drive protein–protein interactions.4 The fact that all proteins are composed of a very limited number of amino acids renders these structures even more fascinating. 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A series of seven different pentapeptides (I–VII) with the general sequence Ac-XXGWS(OPO_3^{2-})-OH was prepared (Fig. 1). The constant domain was composed of a phosphorylated Ser-residue for binding to Au NP 1, a fluorescent Trp-residue for monitoring the binding interaction and a Gly-residue as a flexible spacer to outdistance the two remaining residues of the variable domain. The residues of the variable domain X were chosen from the various subgroups of amino acids ranging from apolar (Phe (I), Leu (II)), polar neutral (Asn (III), Ser (IV)), anionic (Asp (V)), to cationic (Lys (VI), Arg (VII)) in order to explore the compatibility with the self-assembly process (Fig. 1). Two residues of each amino acid were added to enhance their contribution to the overall properties of the corresponding peptide. Fluorescence titration experiments of the peptides at a fixed concentration of Au NP 1 ([TACN-Zn^{2+}] = 10 μM) and ionic strength of 50 mM in aqueous buffer at pH = 7.0 revealed a high affinity of all peptides, except for VI and VII (Fig. 2a). The shallow curvature of the binding isotherms of the latter peptides, as compared to those of peptides I–V, indicates that these peptides bind Au NP
I–V

by the positively charged Lys- and Arg-residues, which cancels
neutralization of the negative charges of the constant domain
with a much lower affinity. This is tentatively ascribed to the
neutralization of the negative charges of the constant domain
by the positively charged Lys- and Arg-residues, which cancels
the main driving force for binding. For this reason, peptides
were discarded from further studies. For other peptides
I–V surface saturation concentrations (SSCs) ranging from
3.1 to 4.3 μM were determined (Table S2, ESI†). Considering
that the monolayer of Au NP 1 with a diameter of 1.8 ± 0.4 nm
contains around 70 thiols, this implies that at saturation
around 20–30 peptides are bound simultaneously to the
monolayer. This indicates that truly multivalent systems are
spontaneously formed. The dynamic nature of these systems
emerged from competition experiments in which the addition
of an increasing amount of adenosine diphosphate (ADP)
resulted in the progressive displacement of peptides from
Au NP 1. The ADP-concentration required to displace 50% of
peptide was used as a value to assess the relative affinities of
peptides I–V for Au NP 1 (Table S2, ESI†). Interestingly, whereas
all peptides have an intrinsic high affinity for Au NP 1 because
of the phosphoSer residue, it turns out that the variable domain
caused significant variations. Peptides with apolar residues
like Phe (I) or Leu (II) had higher affinities compared to those
with polar residues Asn (III) and Ser (IV), which is in line with
previous studies that hydrophobic interactions also contribute
to the interaction between external agents and Au NPs.15 Not
surprisingly, the highest affinity for Au NP 1 was observed for
peptide V, which has two additional negative charges (Fig. 2b).

Having established the nature of the interactions between
the individual peptides and Au NP 1 we then proceeded with
the study of systems of higher complexity. An equimolar
mixture of peptides I–V was prepared and titrated to Au NP 1
([TACN-Zn²⁺] = 10 μM) yielding a binding isotherm similar to
that observed for the separate binding isotherms, which
demonstrates that different peptides can be accommodated
simultaneously on Au NP 1 (Fig. 2c). An SSC of 2.4 μM was
determined for the peptide mixture indicating that under these
conditions the surface of Au NP 1 is saturated with around
17 peptides. This value is lower than that observed for pure
peptides, which suggests that packing is less efficient when
different peptides co-assemble on the surface. In any case,
the high complexity of the self-assembled system emerges from
the calculation of the number of different peptide surfaces that
are created upon the addition of the peptide mixture. Assuming
that all binding sites are identical and independent, the total
number of different combinations, C, is given by

\[ C = \frac{(N+1) \times (N+2) \times \cdots (N+k-1)} {2 \times 3 \times \cdots (k-1)} \]  

with N being the number of available (degenerate) binding sites
and k being the number of peptides. This implies that the
addition of peptides I–V (k = 5) to Au NP 1 (N = 17) results in
the spontaneous formation of around \(6 \times 10^3\) different peptide
surfaces. A displacement experiment using ADP confirmed the
dynamic nature of these surfaces. The irregular nature of the
placement curve reflects the different binding affinities of
peptides I–V: addition of incremental amounts of ADP results
first in the displacement of those peptides with lower binding
affinities (Fig. 2d).

We then proceeded with a proof-of-principle study aimed at
demonstrating the responsive nature of the dynamic peptide
surface. However, this first required a methodology to monitor
the surface composition and changes therein, since the fluores-
cence titrations did not provide this kind of information. We
found that ultracentrifugation using PES-membranes with a
10 kDa molecular weight (MW) cut-off permitted the facile
separation of surface bound peptides from nonbound peptides.16
LC/MS-analysis of the filtrate gave the concentration of each
peptide in solution, from which the surface composition
could then be calculated considering that the initial peptide

Fig. 1 Schematic representation of the self-assembly of dynamic nanoproteins.

Fig. 2 (a) Fluorescence intensity at 360 nm as a function of the concentration of peptide I–V added to Au NP 1. (b) Amount of displaced peptide I–V from the surface of Au NP 1 as a function of the concentration of ADP. (c) Fluorescence intensity at 360 nm as a function of the concentration of a mixture of peptides I–V added to Au NP 1. (d) Amount of displaced peptide mixture from the surface of Au NP 1 as a function of the concentration of ADP. Experimental conditions: [TACN-Zn²⁺] = 10 ± 1 μM; [HEPES] = 10 mM, pH 7.0.
concentrations are known. This protocol was then applied to determine the surface composition of peptides I–V on Au NP 1 as a function of the initial peptide concentration. An equimolar mixture of peptides I–V was added to Au NP 1 ([TACN:Zn²⁺] = 50 μM) at five different concentrations a–e (25, 50, 75, 100 and 125 μM – referring to the overall peptide concentrations). Compared to the fluorescence binding studies, a higher concentration of Au NP 1 was necessary to permit detection of the peptides by LC/MS. It is noted that at this higher concentration a significantly higher SSC of 25 μM was determined for the peptide mixture (see Fig. S8, ESI†). After addition, each sample was centrifuged and the filtrate was analyzed by LC/MS in single ion detection mode. For each peptide, the peak area was normalized on the area measured for the same mixture centrifuged in the absence of Au NP 1. Each experiment was repeated 5 times to give the average values and error margins indicated in Fig. 3.

The absence of peptides in the filtrate of sample a (25 μM) confirms that at this concentration all peptides are bound to Au NP 1, which is in agreement with the fluorescence studies. This was further confirmed by a control experiment in which an excess of adenosine triphosphate (ATP) was added to sample a before filtration. ATP is a strong competitor and displaces all peptides from the surface leading to an increase in the concentration of peptides in the filtrate (see Fig. S10, ESI†).

At peptide concentrations higher than the SSC (samples b–e) a competition has to necessarily take place between the peptides for binding to the limited number of binding positions on Au NP 1, thus creating the conditions for observing a spontaneous adaptation of the surface. Indeed, for all these samples the presence of nonbound peptides resulted in their presence in the filtrate (Fig. 3b). Interestingly, a clear differentiation was observed between the peptides as the concentrations increased from 50 to 125 μM. The surface composition became biased towards peptides I and V, which are indeed those peptides with a higher affinity for Au NP 1. At the highest concentration of 125 μM the assembled peptide surface is composed of 30% of I and 70% of V; peptides II–IV are no longer present on the surface of Au NP 1. These experiments demonstrate the ability of the peptide surface to spontaneously adapt to changes in the environment. In this particular case, the result is a shift from a highly complex peptide surface containing all peptides I–V in equimolar amounts to a surface dominated by peptides I (30%) and V (70%).

In conclusion, we have shown that the self-assembly of small peptides on Au NP 1 is an attractive way to create multivalent peptide surfaces. The resulting systems have a size and complexity similar to proteins and are stable at low micromolar concentrations in aqueous buffer. The surface composition can be simply tuned by changing the nature and ratio of the added peptide fragments. The dynamic nature of the surface permits adaptation of the surface to changes in the environment and offers the possibility of developing self-selection protocols. Combined with a covalent fixation methodology, such as the light-triggered immobilization of peptides on gold nanoparticles that we have reported earlier,17 this approach gives a straightforward access to self-selected multivalent peptide surfaces. We envision that this approach may be used for molecular imprinting of the monolayer to create self-selected active sites for the recognition of small biomolecules or for catalysis, and for the self-selection of peptide surfaces able to interfere with protein–protein interactions.

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References