What does make an amyloid toxic: morphology, structure or interaction with membrane?

Christophe Cullin Sophie Lecomte
Spectroscopy and Imaging of membrane active peptides
- **Amyloids**

**Non-toxic:**
- [URE3]
- [PSI]
- [PIN]
- Curli
- PMEL17
- Het-s

**Toxic:**
- Aß (Alzheimer)
- \(\alpha\)-synucleine (Parkinson)
- huntingtin (Huntington)
- prion (PrP)
- Amyloids

Common characters of amyloids

- Toxicity of amyloids

Schema extracted of Williams and Serpell, FEBS, 2011. 278(20), 3905-17
Toxicity of amyloids

Interaction with membrane
HET-s is a prion protein from the filamentous fungi *Podospora anserina*.

HET-s forms amyloid fibers.

The structure has been determined by solid state NMR.

**Parallel β-sheet organisation**

**PFD of HET-s is non toxic in yeast *Saccharomyces cerevisiae***.

*Wasmer et al., 2008. Science, 319, 1523-1526.*
- Toxicity in *S. cerevisiae*

10 mutations

**In vivo** toxicity

**In vitro** structure

Relationship

**β1**

MKIDAI VGRNSAKDIRTEERARVQLGNVVTAAALHGGIRISDQT TNSVETVVGKGESRLIGNEYGGKGFWDN

**β2**

-M- Y- T- Q- Y- LV- D- K- R-

**β3**

**β4**

**m8**
What is the morphology of the toxic amyloids?
WT amyloids forms 5-nm elementary protofibrils twisted in larger fibers.

\[ > \mu \text{m} \]

M8 fibrils forms very short unbranched fibers that seem to assemble laterally.

What is the structure of the toxic amyloids?
- Study of structure/toxicity

ATR-FTIR spectroscopy on Ge crystal

Determine secondary structure

parallel β–sheet

\[
\begin{align*}
1645 \text{ cm}^{-1} \\
1630 \text{ cm}^{-1}
\end{align*}
\]

antiparallel β–sheet

\[
\begin{align*}
1685 \text{ cm}^{-1} \\
1620-1630 \text{ cm}^{-1}
\end{align*}
\]
- Study of structure/toxicity

Non toxic WT

Toxic M8

parallel $\beta$–sheets

antiparallel $\beta$–sheets

- Study of structure/toxicity

- Study of structure/toxicity

Non toxic WT

Parallel β-sheet

antiparallel β–sheets

What are the targets of the toxic amyloids?

Interaction with membrane using membrane models
- Liposomes (LUV)
- Liposomes (LUV)…

Ta et al. BBA Biomembrane, 1818, 2325-2334 (2012).
- Liposomes (SUV) on Ge crystal

Polarized ATR-FTIR

\[ R_{ATR} = \frac{A_{\parallel}(p)}{A_{\perp}(s)} \]
Liposomes (SUV) on Ge crystal

No variation of the secondary structure of non-toxic or toxic amyloids interacting with lipid bilayers was observed.

Ta et al. BBA Biomembrane, 1818, 2325-2334 (2012).
- Liposomes (SUV) on Ge crystal

ATR-FTIR spectra

**WT**: Ap/As ≈ 1.3 → angle ≈ 30 °

**M8**: Ap/As ≈ 1.6 → angle ≈ 50 °

Ta *et al.* BBA Biomembrane, 1818, 2325-2334 (2012).
Bilayers on PWR crystal

Plasmon Waveguide Resonance spectroscopy

PWR method is similar to SPR but we used polarized (s or p) light. We then obtain information on the orientation of the perturbation.

Harté et al. ChemComm.50, 4168-4171 (2014)
Bilayers on PWR crystal

Plasmon Waveguide Resonance spectroscopy

Addition of WT induces a small positive shift for both polarization, revealing an increase of the refractive index due to a mass increase.

Ta et al. BBA Biomembrane, 1818, 2325-2334 (2012).
- Bilayers on PWR crystal

Plasmon Waveguide Resonance spectroscopy

We observe: large decrease in the spectra depth
increase of the spectral width
2 resonance minimum.

Ta et al. BBA Biomembrane, 1818, 2325-2334 (2012)
We observe: large decrease in the spectra depth, increase of the spectral width, and 2 resonance minimum.

**Toxic peptide induces a lateral membrane reorganization that leads to the formation of two domains.**
- Cryo-TEM

Ta *et al.* BBA Biomembrane, 1818, 2325-2334 (2012).
- Structure-Toxicity study of Aβ

**Aβ<sub>1-42</sub>**

**WT**: D A F R H D S G Y E V H H Q K L V F F A E D V G S N K G A I I G L H V G V V I A

**L34T**: D A F R H D S G Y E V H H Q K L V F F A E D V G S N K G A I I G T H V G V V I A

**G37C**: D A F R H D S G Y E V H H Q K L V F F A E D V G S N K G A I I G L H V C G V V I A
- Structure-Toxicity study of Aβ

Conclusion

The toxicity of amyloid:
morphology, YES
  Short fibers,
structure YES
  Anti parallel β-sheets,
or interaction with membrane? YES
  Toxic mutants interact strongly with membrane.
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MERCI

s.lecomte@cbmn.u-bordeaux.fr